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TRISTEZA DISEASE OF CITRUS [1]

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INTRODUCTION

Tristeza, a serious citrus disease that occurs in all the major citrus-growing districts of Brazil, Argentina, and Uruguay, now seems similar to or identical with serious disorders of sweet orange (*Citrus sinensis* (L.) Osbeck) on sour orange (*C. aurantium* L.) rootstock reported from the Union of South Africa, Java, Australia, and southern California. Methods of transmitting this disease and the susceptibility of various species and varieties of citrus were studied cooperatively in Campinas, Brazil, from 1946 to 1948, symptoms of the disease were observed, and the methods of control tried in Brazil were evaluated. The results of these studies, preliminarily reported in 1947 (*1*), [3] are given herein, together with the history of the disease, the causes assigned to it, and a discussion of scion-stock relation to the disease and the possibility of virus strains.

HISTORY AND CAUSE OF TRISTEZA

The effects of the disease now known as tristeza in Brazil and as podredumbre de las raicillas in Argentina and Uruguay probably have been known for more than half a century. However, only after the appearance of the disease in the citrus-producing areas of Argentina and Brazil, where it caused heavy losses to the citrus industries,

[1] Received for publication July 26, 1948. Part of a cooperative study between Instituto Agronômico and Division of Fruit and Vegetable Crops and Diseases. The study is being continued by Theodore J. Grant, Division of Fruit and Vegetable Crops and Diseases, and the junior author. One important phase of the work will be the testing of all available varieties and species of citrus and citrus relatives for susceptibility to infection and to injury by tristeza when they are used as rootstocks for sweet orange, grapefruit (*Citrus paradisi* Macfad.), and other citrus types of commercial importance.

[2] The writers are greatly indebted to José Pio Nery, chemist, Instituto Agronômico, for making starch determinations for some of the experiments; to the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, for identification of the leafhoppers except *Agallia albidula* Uhl. used in transmission tests; to R. C. Dickson, University of California Citrus Experiment Station, for identification of the aphids; and to Sílvio Moreira, chief of the Department of Horticulture of the Instituto Agronômico, for use of certain unpublished data and for the privilege of making observations on the variety collections and rootstock tests at the Citrus Experiment Station near Limeira (Estação Experimental de Citricultura de Limeira) and for many other kindnesses during the course of these studies.

[3] Italic numbers in parentheses refer to Literature Cited, p. 236.

was it recognized as a distinct disease. Not until its potentialities for causing destruction were fully appreciated were extensive efforts made to determine its nature and control.

The disease appears to have originated in the Union of South Africa, supposedly in some plant native to that country. Early in the development of the citrus industry there, it was found that varieties of sweet orange did not thrive when grafted to sour orange and soon sour orange was abandoned as a rootstock (*35*). Later, efforts to reintroduce sour orange as a rootstock met with failure. Trees of the scion-stock combinations adopted for general use, largely sweet orange on rough lemon (*Citrus limon* (L.) Burn. f.), showed no injury, although there is now reason to believe that the causal agent of the disease persisted in the citrus groves and that infected material was carried to other parts of the world, thus establishing new centers of the disease.

A disease similar to the South African trouble was noted in Java in 1928, when efforts were made to use sour orange as a rootstock for varieties of sweet orange (*33*).

Tristeza was discovered in Argentina in 1930 or 1931 (*9*, *10*). It was found in the Paraíba Valley of Brazil about 1937 (*4*) and was observed in Uruguay in 1940 (*3*).

In 1939 a similar disease was found in a citrus grove in California (*12*). Apparently on account of the rapid decline of affected trees in the first plantings in which it was observed, this disease was called quick decline.

A disease, designated as bud-union decline, which caused decline and death principally of trees of sweet orange and grapefruit on sour orange rootstock was observed in Victoria, Australia, in 1941 (*16*). By 1947 it had caused considerable damage to the citrus industry of that State.

For many years the failure of trees of sweet orange on rootstock of sour orange to thrive in the Union of South Africa was considered to be due to a type of incompatibility between stock and scion. The same condition or a similar one discovered in Java was considered by Toxopeus (*33*) to be due to a type of scion-stock incompatibility in which the sweet orange scion produced a substance toxic to the sour orange stock. After tristeza was discovered in South America, many theories were put forth to explain the effects produced. These include scion-stock incompatibility, soil acidity, various types of nutrient deficiencies, soil toxins, high soil-moisture content, prolonged dry periods, nematode infestations, pathogenic organisms, and infection by a virus.

Bitancourt (*5*) stated that, as a result of observations made on podredumbre de las raicillas in the Province of Corrientes, Argentina, in 1937, he and Fawcett had suggested that one of the possible causes of the disease might be a virus latent in the sour orange and injurious to the sweet orange top. In the same paper Bitancourt explained that the most acceptable hypothesis as to the cause of the disease is that an infectious agent is involved. In 1943 Webber (*35*) proposed the hypothesis that the disease is caused by a virus latent in sweet orange but lethal to sour orange and that regularly and normally the foliage of sour orange and lemon produces some product that inhibits

the action of the virus. In 1944 Bitancourt (*7*) presented the theory that the disease is caused by a virus which is latent in the sweet orange and from which the sour orange is completely immune.

The first experimental evidence supporting the hypothesis that tristeza is caused by a virus was obtained by Meneghini (*18*), who reported in 1946 that the disease is transmitted by an aphid. The vector was identified tentatively as *Aphis tavaresi* Del Guercio; but probably it is more properly called *A. citricidus* (Kirk.), for which *A. tavaresi* and *A. citricola* Van der Goot appear to be synonyms. In 1947 Bennett and Costa (*1*) reported the confirmation of Meneghini's transmission results with *A. citricidus*. They reported also transmission of the disease in greenhouse and field tests in which buds and twigs from diseased trees were grafted to sweet orange plants.

Fawcett and Wallace (*13*) in 1946 and Wallace and Fawcett (*34*) in 1947 reported transmission of quick decline in California by buds from diseased trees, and Oberholzer (*21*) in 1947 reported results indicating bud transmission of the disease that causes failure of trees of sweet orange on sour orange rootstock in the Union of South Africa.

It now seems well established that tristeza in Brazil is caused by a virus that is transmitted by the oriental citrus aphid (*Aphis citricidus*). At certain seasons this aphid is found in abundance on citrus trees in southern Brazil. It occurs also in Argentina and Uruguay; and, according to Takahashi (*31, p. 47*), it has been reported from Japan, China, India, Java, Sumatra, Ceylon, Hawaiian Islands, South America, and Africa. Apparently it has not been found in the United States.

ECONOMIC IMPORTANCE AND SPREAD OF TRISTEZA IN INVADED AREAS

The spread of tristeza in South America, as described by several investigators (*2, 7, 8, 20*), has been relatively rapid. The disease probably now occurs in all the major citrus-producing areas of Brazil, Argentina, and Uruguay. In about 18 years it has killed or rendered of little commercial value a high percentage of the orange trees on sour orange rootstock in these countries.

In the State of São Paulo, Brazil, the disease spread in about 12 years to all the citrus-producing areas, infecting and destroying upward of 6,000,000 trees, or about 75 percent of the orange trees of the State.

The spread of the disease within groves also has been rapid after it became established. For more than 6 years Moreira [4] followed the spread of tristeza in a grove of 2,606 trees of the variety Bahianinha on sour orange rootstock, located at the Estação Experimental de Citricultura de Limeira, and obtained the following results:

Date of observation:	*Trees affected with tristeza*	
	Number	*Percent*
November 28, 1942	308	11. 8
October 8, 1943	508	19. 5
January 4, 1945	1, 435	55. 1
January 10, 1946	1, 890	72. 5
January 19, 1948	2, 606	100. 0

[4] Unpublished information made available to A. S. Costa by Sílvio Moreira.

In most groves of sweet orange on sour orange rootstock affected with tristeza large numbers of trees have died after a few years. However, in many instances factors other than tristeza probably are important in the short life of the affected trees. Diseased groves soon become unprofitable and are abandoned. The diseased trees then suffer from weed competition and general lack of care, which contribute materially to the rate of decline. This is indicated by the observations of Moreira in the grove at Limeira already mentioned, where, after more than 6 years during which the trees had good care, only 15.5 percent of the trees had died; at the end of the period, however, none of the trees had commercial value.

SYMPTOMS OF TRISTEZA

The first evidences of tristeza in older orange trees usually are a partial to almost complete suppression of new flushes of growth and the production of various types of leaf discolorations. The first symptoms may appear on only one branch, but often a greater part of the tree is involved. Older leaves become dull or slightly bronzed at first; later they may take on various shades of yellow, the amount of yellowing apparently depending on environmental conditions and on the variety involved. The Barão variety of sweet orange appears to be more subject to leaf yellowing than some other varieties. Yellowing may occur uniformly over an entire leaf, or it may be more conspicuous in some parts than in others; often it is more intense in the midrib or in the midrib and lateral veins. Occasionally diseased leaves tend to stand upright. Diseased trees with yellow leaves are conspicuous for considerable distances.

Soon after leaf discoloration begins, the older leaves at the base of the twigs begin to fall. Usually leaf fall continues progressively toward the tip until many of the twigs are defoliated or have only a few of the younger leaves remaining. In many cases abscission takes place between the petiole and the leaf blade, the petiole remaining attached to the twig for a time after the blade has dropped. As leaf fall continues, the limbs of the tree become exposed. Weak shoots begin to grow out of axillary buds, mostly in a vertical position; these shoots produce small leaves, paler than normal ones and sometimes with yellow midribs. Limbs begin to die back from the tips, and more weak shoots are produced from the main limbs and trunk. After a tree has shown symptoms for 2 to 3 years, in most cases nearly all the smaller limbs are dead and the tree is devoid of foliage except for a few weak shoots on the main limbs and trunk. Under average conditions twig growth usually becomes weaker each season until the tree dies. Some trees look, however, as if they might remain alive indefinitely, with a reduced number of weak shoots.

Decline, however, does not follow this general pattern in all cases. Some trees collapse quickly, but such collapse appears to be relatively rare. Others decline slowly and finally reach a stage at which they produce sparse foliage, more or less normal in color, relatively few blossoms, and light crops of fruit of inferior quality. Some affected trees seem capable of persisting indefinitely in this state of reduced vigor.

Diseased trees have a tendency to blossom heavily in the earlier stages of attack and usually set heavy crops of fruit. When such fruits begin to take on color, they are more conspicuous than those on healthy trees, partly because of the smaller amount of foliage on the diseased trees.

Trees with symptoms of tristeza in the tops also show severe root injury. There is a marked depletion of starch in the earlier stages in the rootlets and later in the larger roots. First the rootlets die and decay, and later the injury extends to the larger roots until the capacity of the tree to take up water and mineral nutrients is greatly impaired. In general, top symptoms on larger trees are those that may be produced as a result of root injury.

Bitancourt (*7*) found also that in diseased trees there is evidence of a greater concentration of starch in the outer bark of the sweet orange scion immediately above the graft union than in the outer bark of the sour orange stock. Rossetti (*25*) found this condition to prevail in most of the healthy-appearing trees as well as in obviously diseased ones of susceptible scion-stock combinations at the Estação Experimental de Citricultura de Limeira. It has not been determined, however, that starch concentration in the bark can be used to detect infection well in advance of the production of symptoms in the foliage.

Starch depletion from the roots and accumulation in parts of the tops of trees appear to be associated with necrosis in the phloem, shown by Schneider, Bitancourt, and Rossetti (*29*) to occur in the bark of diseased trees in the region of the bud union. A similar type of necrosis in trees affected with quick decline was described by Schneider (*27*).

It has not been feasible as yet to determine the incubation period of the disease on larger trees. On young trees in nursery rows symptoms

FIGURE 1.—Orange variety Bahianinha on sour orange rootstock photographed 10 months after inoculation: *A*, Noninoculated (check) trees (first five in row); *B*, trees inoculated by insertion of buds from diseased sweet orange trees (first five in row).

have been observed occurring naturally 18 months after budding. Trees 1 to 2 years old may produce symptoms 4 months after inoculation, and small plants in pots produce yellow leaves in periods as short as 30 days. Symptoms in small trees vary greatly, but usually affected plants are somewhat unthrifty in appearance and the leaves range in color from slightly more dull than normal to yellow. Diseased trees may blossom and set a crop of fruit the first year after being transplanted from the nursery (fig. 1). Symptoms on young trees are described in greater detail on pages 213, 217, and 219.

TRANSMISSION OF TRISTEZA

Attempts were made to transmit tristeza of citrus to nursery-grown orange trees, to small potted sweet orange trees on sour orange rootstock in the greenhouse, and to various other species of plants, mostly annuals, that were readily available. Methods of transfer included grafting and budding from diseased to healthy orange trees, inoculation with juice from diseased orange trees, training dodder from diseased to healthy plants, and infesting plants with various species of insects found to feed on affected trees.

INOCULATION OF NURSERY-GROWN TREES BY BUDDING

In the first test of transmission of tristeza to trees in the field, 20 trees of Bahianinha sweet orange on sour orange rootstock were selected from a nursery near Limeira and planted in plots at Campinas on October 31, 1946. Ten of these plants were inoculated on November 20 by inserting 2 buds from diseased trees in each. The trees were reinoculated on December 18. The remaining 10 trees were held as checks. Because these trees came from an area where tristeza was prevalent, some of them may have been infected naturally before inoculation.

One of the check trees began to produce yellow leaves in mid-January and showed other symptoms characteristic of tristeza. By June 1, 1947, the 10 inoculated trees and 3 of the check trees showed rather marked symptoms of tristeza. All the diseased trees blossomed, and 9 of the 10 inoculated trees set fruit which matured in early September (fig. 1, *B*). All the inoculated trees and the 3 diseased check trees blossomed heavily again in August and early September. By October 1 the 7 healthy-appearing check trees were considerably larger than the inoculated trees, had normal green foliage, had blossomed sparsely, and had set no fruit. By March 30, 1948, 1 of the 10 inoculated trees had died; 9 were markedly stunted in comparison with the check trees, but 8 of these showed some degree of recovery in the leaves. Of the 10 check plants, 4 showed some stunting without chlorosis and 1 of the larger trees was beginning to produce chlorotic leaves in which vein yellowing was evident.

In a second test 5 to 25 trees of each of 11 varieties of sweet orange budded on sour orange rootstock which had been obtained from a nursery near Santa Rita during the early part of December 1946 were planted in plots at Campinas (table 1). At the time the trees were selected tristeza was beginning to appear in some of the commercial groves at Santa Rita, but since the majority of the citrus trees appeared

healthy it was hoped that most of the young trees selected would prove to be free of infection. At least half of the trees of each variety were inoculated by insertion of 2 buds from diseased trees into each tree on January 18, 1947, and were reinoculated with the same number of buds on April 9. There was a high mortality in the buds of the first inoculation, but those of the second survived reasonably well. No buds were inserted into the check trees.

TABLE 1.—*Results of inoculating nursery trees of sweet orange on sour orange rootstock by means of buds from diseased trees, Campinas, Brazil, 1946–47*

[Test 1: Trees planted Oct. 31, 1946, inoculated Nov. 20 by insertion of 2 buds from diseased trees into each tree, and reinoculated Dec. 18 with buds from same source. Test 2: Trees planted during first week of December 1946, inoculated Jan. 18, 1947, and reinoculated Apr. 9. Results recorded Oct. 1, 1947]

Test and variety [1]	Trees inoculated	Inoculated trees diseased	Check trees	Check trees diseased
Test 1:	*Number*	*Number*	*Number*	*Number*
Bahianinha	10	10	10	3
Test 2:				
Abacaxí	5	0	5	0
Bahianinha	5	5	0	
Barão	15	10	10	0
Campista	5	5	5	0
Coronel	5	5	5	1
Lima	5	4	5	0
Mangaratiba	5	3	5	0
Parnaso	5	5	5	0
Pera do Rio	10	9	10	0
Seleta	15	13	10	0
Serrana	5	4	5	0

[1] Varieties are listed by the names under which they were received from the nursery. These trees were inspected in early 1948 by Sílvio Moreira, and it is his opinion that the varieties labeled Coronel and Lima are Laranja Serra Dagua and Serrana de Limeira, respectively, and that the variety labeled Campista is Laranja Coco.

By the first of May, or about 3½ months after the first inoculation, some of the inoculated trees of the variety Barão began to show an abnormal coloration of older leaves, consisting chiefly of a dull appearance with a suggestion of vein yellowing. About a month later some of the inoculated trees of the variety Seleta began to show abnormal coloration of the foliage. Symptoms considered to be caused by tristeza appeared in other varieties, and by October 1 most of the inoculated trees of all of the varieties except Abacaxí appeared to be diseased (table 1). Abnormalities consisted chiefly of a certain degree of dullness of the older leaves and of various types and degrees of yellowing of the foliage including some vein yellowing. As symptoms increased in severity the plants became slightly stunted, new growth was partially suppressed, and many of the diseased trees blossomed heavily.

Leaf cast occurred to some extent on some of the trees of the varieties Barão and Mangaratiba, and it was especially heavy on all the inoculated trees of the variety Coronel (fig. 2). There was considerable variation among the varieties in the degree and type of yellowing of the foliage. Vein yellowing was more evident in mature leaves of the variety Barão than in those of other varieties. Some vein yellowing was evident also in the leaves of the Mangaratiba, but the most outstanding characteristic on this variety was the rather uniform yellowing of the leaves on some of the younger shoots. Leaf dropping was followed by the production of short, weak shoots along the main

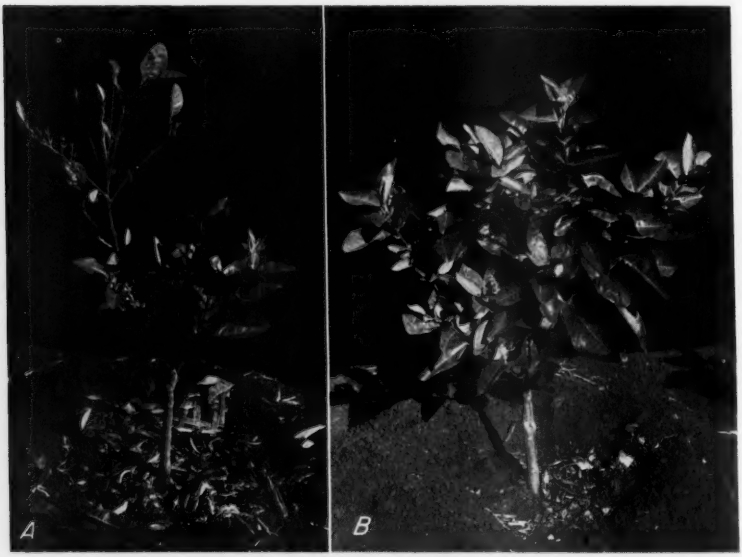

FIGURE 2.—Young orange trees of the variety Coronel on sour orange rootstock planted in field at Campinas, Brazil, during the early part of December 1946 and photographed about 7 months after inoculation: *A*, Tree inoculated by insertion of buds from diseased sweet orange trees on January 18, 1947; *B*, noninoculated (check) tree.

limbs. Except for one tree of Coronel, which lost many of its leaves, blossomed heavily, and was obviously diseased, none of the check trees showed symptoms characteristic of tristeza at the time of these observations.

Tests were made at intervals on inoculated and noninoculated groups of plants of this experiment by Bitancourt's method (*7*) to determine the relative amounts of starch in the outer bark of the sweet and the sour orange in the vicinity of the graft union. In some cases results of the starch test correlated reasonably well with the conditions of the tops, but in most cases they were erratic. By the method suggested by Fawcett (*11*) for quick decline, tests were made to determine starch content of the roots of all the trees of this experiment about 6 months after inoculation and about 2 months after some of the trees had begun to show symptoms of the disease in the tops. No marked evidence of starch depletion was observed in the roots of inoculated plants at that time; however, it is presumed that starch depletion will take place in these plants as the disease progresses.

Iodine tests made about 6 months after inoculation indicated that leaves of inoculated plants had more starch than those of noninoculated plants. Starch determination [5] showed that in two samples of Bahianinha leaves the starch content averaged 0.742 and 0.346 percent, respectively, for inoculated and noninoculated trees and in three

[5] Made by José Pio Nery.

samples of Seleta leaves, 1.192 and 0.607 percent, respectively, for inoculated and noninoculated trees. One determination gave 0.920 and 0.373 percent starch, respectively, in leaves of girdled and nongirdled limbs of a healthy Bahianinha tree, indicating that starch accumulation in the leaves of girdled healthy twigs may be of about the same order as that in diseased orange trees in certain stages of disease. Discoloration of the leaves of the girdled branches was more or less similar to that of leaves of some of the inoculated trees in this test.

INOCULATION OF GREENHOUSE PLANTS

An extensive series of inoculation tests was made in greenhouses screened to give a considerable degree of protection against insects. Most of the inoculations were made on small, rapidly growing, potted plants of sweet orange on sour orange rootstock. The plants used in these tests were obtained by grafting sweet orange seedlings on sour orange seedlings, by a method already described (*1*), when the respective seedlings were 6 to 10 cm. tall.

Juice Inoculations

Succulent twigs from badly diseased trees of sweet orange on sour orange rootstocks were selected to serve as sources of inoculum for juice inoculations. The twigs were ground in a meat chopper, and the sap was extracted from the macerated leaf and twig material with a small press and used immediately. Inoculations were made by first sprinkling the plants with an abrasive and then rubbing the leaves with a cloth pad saturated with inoculum.

In 1 experiment 40 vigorously growing orange plants about 15 cm. tall were inoculated. None of these plants showed symptoms of tristeza after 4 months. The 40 check plants also remained healthy. In a second experiment several methods of mechanical inoculation were employed in inoculating 50 small plants of the variety Barão on sour orange rootstock, but none of these and none of the 50 check plants of this test showed evidence of infection.

In addition, 67 species and varieties of plants of various types were inoculated in an effort to find a plant susceptible to infection by juice inoculation. Most of these species were annuals grown in pots from seeds. Twenty-five or more plants of most kinds were inoculated when relatively small and growing rapidly. No symptoms considered to be caused by the virus of tristeza were observed on any of these plants.

Tests for Dodder Transmission

During the past few years several viruses have been transmitted by dodder (*Cuscuta* spp.). This method of transmission has been particularly successful with viruses that cause yellowing or leaf curling and that appear to have a close association with the phloem of the host plant. Evidence of phloem collapse presented by Schneider, Bitancourt, and Rossetti (*29*) suggests that tristeza may be caused by a virus of this type, but this evidence alone is not sufficient to justify final conclusions.

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Five species of dodder (*Cuscuta americana* L., *C. campestris* Yuncker, *C. indecora* Choisy, *C. subinclusa* Durand and Hilgard, and *Cuscuta* sp. collected near Campinas) were used in transmission tests. All these except *C. campestris* grew well on orange trees. In the inoculation tests the dodder was first established on succulent shoots of diseased orange trees growing in barrels in the greenhouse. At intervals over a period of about 7 months stems of dodder were trained from the diseased trees to healthy plants of various kinds. After about 7 days the stems connecting the diseased trees with the inoculated plants were broken. The inoculated plants were kept under observation for several months. The species and varieties of plants inoculated and the results obtained are presented in table 2.

TABLE 2.—*Results of attempts to transmit tristeza virus by training dodder from infected orange trees and allowing it to become established on healthy plants of various kinds*

Species of dodder (*Cuscuta*) tested	Plant inoculated [1]	Plants inoculated	Plants infected
		Number	*Number*
C. americana	*Citrus sinensis* (sweet orange)	20	0
	Daucus carota L. (carrot)	13	0
	Datura stramonium L. (jimsonweed)	12	0
	Euphorbia prunifolia Jacq. (amendoim bravo)	6	0
	Lycopersicon esculentum Mill. (tomato)	10	0
	Nicotiana glauca Graham (tree tobacco)	4	0
	Nicotiana glutinosa L.	5	0
	Nicotiana tabacum L. (tobacco, var. Turkish)	10	0
	Phytolacca americana L. (pokeweed)	14	0
	Sida rhombifolia L.	5	0
C. campestris	*Citrus sinensis* (sweet orange)	11	0
	do	20	0
C. indecora	*Lycopersicon esculentum* (tomato)	16	0
	Nicotiana glauca (tree tobacco)	6	0
	Phytolacca americana (pokeweed)	8	0
	Vinca rosea L. (periwinkle)	15	0
C. subinclusa	*Citrus sinensis* (sweet orange)	20	1
	Beta patellaris Moq	19	0
	Citrus sinensis (sweet orange)	20	1 (?)
	Daucus carota (carrot)	14	0
	Lycopersicon esculentum (tomato)	20	0
Cuscuta sp.	*Nicotiana glutinosa*	11	0
	Nicotiana tabacum (tobacco, var. Turkish)	9	0
	Phytolacca americana (pokeweed)	12	0
	Physalis sp. (S–51)	20	0
	Physalis sp. (S–92)	6	0
	Vinca rosea (periwinkle)	6	0

[1] All plants of *Citrus sinensis* were on sour orange rootstock.

None of the noncitrus plants used in these tests showed evidence of disease. However, one of the sweet orange plants on which *Cuscuta subinclusa* was established developed yellowing and stunting typical of plants affected with tristeza. Virus was transmitted from this plant to other orange plants by means of *Aphis citricidus*. Also one sweet orange plant on which *Cuscuta* sp. was established began to produce yellow leaves after about 2 months. Yellowing was still evident after 3 months and the plant was stunted, but no attempts were made to recover tristeza virus. Of 20 check plants of sweet orange on which dodder (*Cuscuta subinclusa*, 10 plants; *Cuscuta* sp., 10 plants) from healthy orange plants was established, none showed symptoms of tristeza after 6 months.

Further tests with larger numbers of plants are needed before definite conclusions may be drawn regarding transmissibility of the tristeza virus by dodder. However, the available evidence indicates

that the virus may be transmissible to a low percentage of sweet orange plants by certain species. Transmission to a low percentage of plants or to no plants would indicate that dodder is not a host of the tristeza virus.

Transmission by Tissue Union

Attempts were made to transmit tristeza to small potted plants of sweet orange on sour orange rootstock by means of buds and twig grafts. In the attempts to transmit the disease by budding, vigorously growing plants 30 to 60 cm. tall were inoculated by inserting one small bud from a diseased plant in the sweet orange scion of each healthy plant a short distance above the graft union with the sour orange rootstock. Of 40 plants inoculated with buds in this manner, 15 later showed symptoms characteristic of tristeza; whereas, 40 plants of the same lot not budded remained healthy.

Two methods of inoculation by grafting twigs from diseased plants to healthy ones were used. In the first method potted sweet orange plants 30 to 50 cm. tall were placed adjacent to diseased plants growing in pots or barrels. A downward incision was made in the stem of the plant to be inoculated, and a similar but inverted incision was made in an adjacent twig of the diseased plant. The V-shaped segment of stem of the diseased twig was fitted into the incision of the healthy plant, and the two twigs were bound together firmly with a cord. After about 30 days each diseased twig was severed just below the new graft union. Of 26 plants inoculated in this manner, 21 showed definite symptoms of tristeza. Of the 10 check trees grafted with twigs from healthy plants, none showed evidence of infection.

In the second method of inoculation suitable twigs were taken from diseased trees and grafted to the stems of potted plants by the method just described. The lower ends of the twigs were kept in vials of water for 30 days or more. After union was complete the stems were severed just below the new graft union. In three separate tests this method of inoculation resulted in high percentages of infection. Of 15 plants inoculated, 14 showed symptoms of tristeza. Of 15 check plants of the same lot not grafted with twigs, all remained free of symptoms.

Plants grafted with twigs that remained attached to diseased trees for 30 days or more showed symptoms of disease in a relatively short time. Usually within 30 days after inoculation new leaves at the tips of the plants began to show a pale-yellow color. Further growth resulted in more yellow leaves, and in some cases the older leaves of the plants also became yellow; in other plants the older leaves did not turn yellow but were somewhat less glossy than those of healthy plants. Stunting was marked regardless of the degree of yellowing of the foliage. After 4 to 6 months the check plants were two or three times as tall as the inoculated ones.

Plants inoculated by budding or by grafting with detached twigs were slower in showing symptoms, and the incubation period of the disease varied from 1 to 4 months. After symptoms began to appear, however, they were similar to those produced by other methods of inoculation.

Tests for Insect Transmission

The species of insects tested for ability to transmit the virus of tristeza are listed in table 3. Of these species, *Aphis citricidus*, *A. gossypii* Glov., *Macrosiphum solanifolii* (Ashm.), and *Aleurothrixus floccosus* (Mask.) were found breeding on citrus trees under field conditions. All the other species except *Macrosiphum ambrosiae* (Thos.), *Myzus persicae* (Sulz.), and *Aphis rumicis* L. were found on orange trees at different times, but it was not determined that any of them breeds on citrus. In all the tests except those with *A. citricidus* the insects were collected and placed on diseased trees on which they were allowed to feed for periods ranging from several hours to several days. The insects were then transferred to healthy plants. Large numbers of insects were used in most of the tests.

Table 3.—*Results of tests in which sweet orange plants on sour orange rootstock were infested with different species of insects from diseased orange trees to determine whether they are vectors of tristeza virus* [1]

Insect tested	Source of insect	Plants infested	Plants infected
		Number	*Number*
Aphis citricidus	Diseased orange tree	20	20
	Nonviruliferous colony	20	0
	Diseased orange tree	5	4
	do	10	8
	Nonviruliferous colony	10	0
	Diseased orange tree	38	22
	Nonviruliferous colony	20	0
	Diseased orange tree	10	7
	do	10	10
	Nonviruliferous colony	10	0
Aphis gossypii	Diseased orange tree	15	0
Aphis rumicis	do	13	0
Macrosiphum ambrosiae	do	5	0
Macrosiphum solanifolii	do	15	0
	Potato	5	0
Myzus persicae	Diseased orange tree	10	0
Aleurothrixus floccosus	do	10	0
Agallia albidula	do	11	0
Agalliana ensigera Oman	do	10	0
Bucephalogonia sp.	do	10	0
Empoasca batatae Poos	do	10	0
	Sweetpotato	5	0
Miscellaneous leafhoppers	Diseased orange tree	5	0

[1] The number of insects used to infest the test plants ranged from 15 to 50 for *Bucephalogonia* sp. and from 50 to 300 for all other species, the larger numbers being used on plants infested with the different species of aphids.

In the tests with *Aphis citricidus*, infested twigs were taken from diseased orange trees and placed directly on healthy plants. The insects crawled to the healthy plants as the diseased twigs wilted. The aphids were allowed to feed 2 to 4 days and were then killed with a nicotine spray. Check plants were infested with aphids from healthy orange trees in cages. These aphids were the progeny of individuals taken from their mothers at birth and placed on healthy seedling plants. Except as noted, 100 to 300 aphids were placed on each infested plant.

A high percentage of infection was obtained in each experiment in which *Aphis citricidus* was used, but no evidence of infection was observed on plants infested with other species of insects. Some of the plants infested with *A. citricidus* began to produce yellow leaves

at the growing tips 30 days after infestation. After these leaves began to appear growth was much retarded and old and young leaves showed various degrees of yellowing. The leaves of some of the diseased plants, especially those of the variety Barão, had a tendency to stand more nearly upright than those of healthy plants. On some plants the leaves turned bright yellow, and in a few cases they dropped. Yellowing of other plants was less marked, and the leaves were only slightly paler than normal. Some of the plants of this latter type produced new shoots with small leaves more or less normal in color. In general, the yellow plants were smaller than those with greener foliage. All the check plants that were infested with large numbers of aphids from nonviruliferous colonies remained healthy and grew rapidly. Figure 3 shows an infected yellow plant and a check plant of one experiment about 6 months after the plants were infested.

One test was made to obtain evidence as to whether relatively small numbers of *Aphis citricidus* can effectively transmit tristeza and whether a period of starvation before feeding on diseased plants increases the infectivity of the aphids. For this test, aphids were taken from the nonviruliferous colonies and placed on wet filter paper in moist chambers. After a starvation period of 4 to 7 hours the aphids were removed and placed on succulent twigs from diseased sweet orange plants on which they were allowed to feed for periods ranging from 5 to 60 minutes before being transferred to healthy plants. Each plant was infested with 50 aphids. As one of the controls, each plant was infested with 50 aphids taken directly from diseased plants; as a second control, on relation of numbers of insects per plant to infection, each plant was infested with about 300 aphids taken directly from diseased plants. The feeding periods and the results are shown in table 4.

TABLE 4.—*Results of transmission tests with Aphis citricidus in which nonviruliferous aphids were starved for periods of 4 to 7 hours, allowed to feed for indicated periods on diseased sweet orange twigs, and then transferred to small plants of sweet orange on sour orange rootstock*

Source of aphids	Periods starved aphids fed on diseased plants	Aphids per plant	Plants infested	Plants infected
	Minutes	*Number*	*Number*	*Number*
Nonviruliferous colony	0	50	5	0
Do	5	50	5	0
Do	10	50	5	0
Do	30	50	5	0
Do	60	50	5	1
Diseased orange trees	(1)	50	5	3
Do	(1)	300	5	5

[1] Aphids reared on diseased plants, not starved; transferred directly from diseased twigs to healthy plants.

This test is too limited to permit the drawing of final conclusions, but the results indicate that the starvation period prior to feeding on the diseased plants did not result in greater efficiency in transmission. They suggest also that feeding periods of 60 minutes or more are required for the insect to become fully charged with virus. Fifty aphids from a diseased tree appeared less effective in transmitting the virus than 300 aphids.

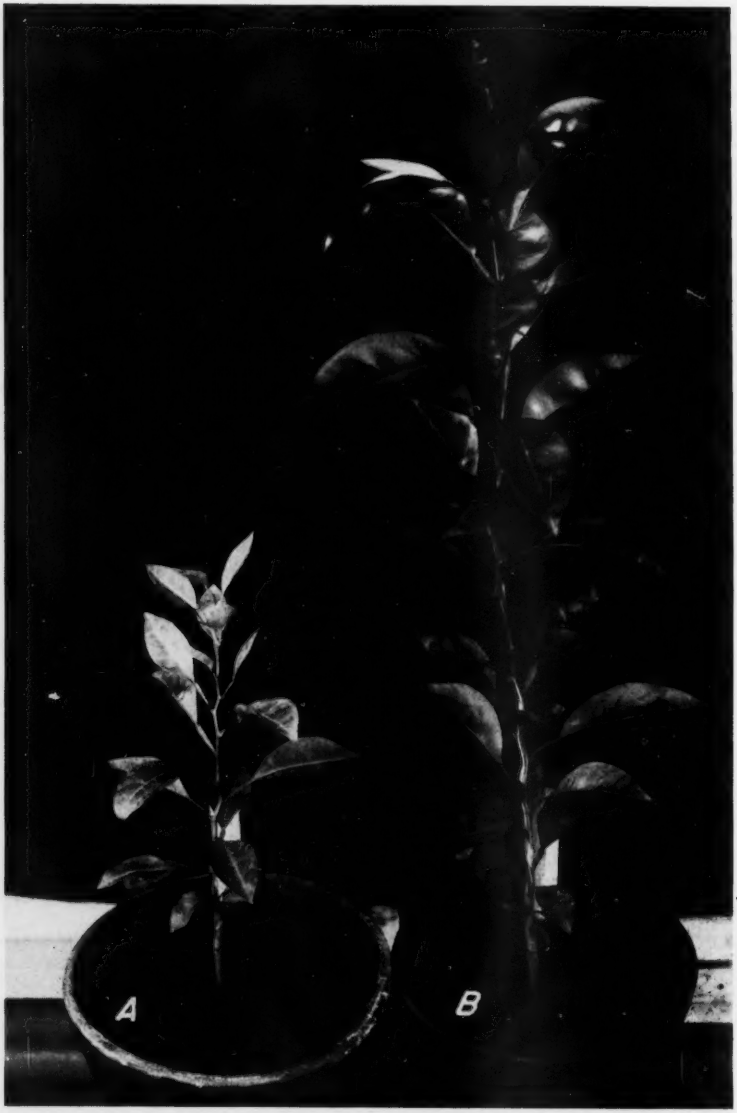

FIGURE 3.—Young sweet orange trees on sour orange rootstock photographed about 6 months after inoculation: *A*, Plant inoculated by infestation with about 200 *Aphis citricidus* from a sweet orange tree affected with tristeza; *B*, check plant infested with about the same number of aphids from healthy plants.

In two other tests different numbers of aphids collected from diseased plants were used to inoculate very small plants of the variety Barão on sour orange rootstock. Of 30 plants each infested with 1 aphid, 2 became infected; of 5 plants each infested with 5 aphids, 1 became infected; of 2 plants each infested with 25 aphids, 1 became infected; and of 4 plants each infested with many aphids, 1 became infected.

Other tests were made in which large numbers of *Aphis citricidus* were allowed to crawl from diseased orange twigs to small rapidly growing plants of various species and remain until they died. Ten to fifty plants of each of the following species were infested in this manner: *Acanthospermum hispidum* DC. (carrapicho de carneiro), *Amaranthus caudatus* L., *Brassica pe-tsai* Bailey (Chinese cabbage), *Callistephus chinensis* (L.) Nees (China-aster), *Canavalia ensiformis* (L.) DC. (feijão de porco), *Datura stramonium* (jimsonweed), *Datura* sp., *Erigeron bonariensis* L., *Euphorbia pilulifera* L., *E. prunifolia* (amendoim bravo), *Gossypium hirsutum* L. (cotton), *Lycopersicon esculentum* (tomato), *Nicotiana glauca* (tree tobacco), *N. tabacum* (tobacco), *Oxalis* sp., *Phaseolus vulgaris* L. (kidney bean, var. Scotia), *Phyllanthus corcovadensis* Muell. Arg. (quebra pedra), *Ruta* sp., *Solanum tuberosum* L. (potato), *Spinacia oleracea* L. (spinach), *Stellaria media* (L.) Cyr. (chickweed), and *Vigna sinensis* (Torner) Haask. (cowpea). No symptoms considered to be caused by the tristeza virus were discovered in any of these plants.

TESTS FOR SEED TRANSMISSION

All the seedling plants of sweet and sour oranges used in the experiments already described were grown from seeds from diseased trees or from trees that had been exposed to infection for several years. More than 1,200 plants were prepared for these experiments by grafting sweet orange seedlings on sour orange seedlings. Some of the plants were inoculated by insertion of grafts or by infestation with *Aphis citricidus* and showed the disease only after a reasonable period following inoculation. More than 425 plants, however, were held as checks or were inoculated with juice or by infestation with insects that appear not to be vectors of the causal virus. Only 1 of these plants has shown symptoms that resemble those of tristeza. Attempts to recover the tristeza virus from this plant were unsuccessful, and it was concluded that the symptoms observed were not caused by that virus.

Further tests were made with seeds collected from trees of the variety Pera on sour orange rootstock in advanced stages of disease and with seeds from trees of sour orange on their own roots which showed no symptoms of tristeza but which were surrounded by badly diseased trees of sweet orange on sour orange rootstock. Seeds of the two species were planted in flats. When the seedlings attained sufficient size, the sweet orange seedlings were grafted onto sour orange seedlings. More than 1,000 plants were prepared in this manner, placed in pots in a greenhouse, and watched for the appearance of symptoms of tristeza for 5 months. All of them remained healthy. From these results it seems probable that the tristeza virus is not transmissible through the seeds of the varieties of oranges tested.

The causal agent of the tristezalike disease of citrus in the Union of South Africa also appears not to be transmissible through the seed, since Oberholzer (*21*) found that buds from seedlings of Valencia orange and Triumph grapefruit developed normally when budded into sour orange and lemon rootstocks, whereas buds from orchard trees developed into twigs with severe symptoms of disease on these rootstocks.

VARIETAL SUSCEPTIBILITY TO TRISTEZA

The degree of susceptibility of the various species and varieties of citrus to infection with tristeza is not well known, largely because the causal virus had not been found to produce macroscopic primary symptoms on any of the plants that have been subjected to inoculation. Seedling trees of sour orange, sweet orange, rough lemon, Rangpur lime (*Citrus aurantifolia* (Christm.) Swingle, or possibly *C. reticulata* × *C. aurantifolia*), sweet lime (*C. aurantifolia*), grapefruit, "cravo" tangerine (*C. reticulata* Blanco), citron (*C. medica* L.), and trifoliate orange (*Poncirus trifoliata* (L.) Raf.) have been growing at the Estação Experimental de Citricultura de Limeira near diseased trees for more than 5 years and have shown no injury from the disease. So far as known, seedling trees of all species and varieties of citrus are immune from or highly resistant to injury, although some are known to be susceptible to infection. Thus far, damage has been observed only on trees of certain specific scion-stock combinations.

SUSCEPTIBILITY OF SCION-STOCK COMBINATIONS

The scion-stock combination injured most extensively in Argentina, Brazil, and Uruguay is sweet orange on sour orange rootstock. Other kinds of citrus on this rootstock, however, have also been reported as susceptible to injury (*2*, *5*, *6*, *20*). In 1941 Bitancourt (*5*) listed sweet orange as susceptible to injury in Argentina and grapefruit as possibly susceptible; in 1943 he (*6*) reported sweet orange, tangerine, and grapefruit as susceptible in Brazil. Moreira (*20*), in 1942, reported the susceptibility of Pera and Bahianinha sweet orange, Marsh seedless grapefruit, Mexeriqueira tangerine, and Galacian lemon. In 1947 Bertelli (*2*) listed sweet orange, grapefruit, and mandarin as subject to injury.

During the 1946–47 season observations were made on many varieties and species of citrus growing at the Estação Experimental de Citricultura de Limeira, where a high percentage of the trees of sweet orange on sour orange rootstock were in advanced stages of disease. In these plantings most of the trees in blocks of Marsh seedless grapefruit on sour orange and on bittersweet orange (*Citrus aurantium* L.) showed symptoms characteristic of those produced by tristeza. These trees were smaller than those on sweet orange rootstock; many older leaves had dropped prematurely; in some cases weak secondary branches with small leaves had developed on some of the limbs; and the fruits were smaller and probably more numerous than on adjacent healthy-appearing trees on sweet orange rootstock. The trees had not declined in vigor or productivity so rapidly as had orange trees on sour orange rootstock. The symptoms, however,

were those that would be expected from a less severe expression of the disease. This greater resistance of grapefruit has been noted also by Bitancourt (*6*).

Orange trees of the varieties Pera and Bahianinha on grapefruit rootstock also appeared abnormal and showed many of the symptoms characteristic of tristeza. Injury was less severe than on trees of these varieties on sour orange rootstock. It has been suggested that the abnormal appearance may have resulted from a type of incompatibility between scion and stock. However, since there appears to be no evidence of a comparable incompatibility in this union in other parts of the world, it seems possible that these trees are being injured by tristeza.

In variety-test plots a short row of trees of Thornton tangelo (*Citrus paradisi* × *C. reticulata*) on sour orange rootstock had been killed by tristeza, but an adjacent row of Sampson tangelo on the same rootstock showed no evidence of injury. Differences in susceptibility of these two varieties of tangelo were reported first by Moreira (*20*) in 1942.

In experimental tests 30 or more citrus plants of each of several citrus types were prepared in 3 lots as follows: Lot 1, seedlings on their own roots; lot 2, seedlings grafted to sour orange rootstock; lot 3, seedlings grafted with sweet orange scions. Half of the plants of each lot were infested with large numbers of *Aphis citricidus* from diseased orange trees, and half were retained as checks and not infested with aphids. In these tests, which are still in progress, no evidence of disease has been observed on nongrafted trees of any species or variety, but symptoms typical of tristeza have appeared on the following scion-stock combinations: Sweet orange on Viçosa grapefruit and Doce and Melancia shaddocks (*Citrus grandis* (L.) Osbeck); and Viçosa grapefruit, Doce shaddock, Cleopatra mandarin (*C. reticulata*), Satsumelo 10–V–3, and Sunshine tangelo, all on sour orange rootstock. No evidence of disease was noted on any of the noninoculated plants of these tests.

Many scion-stock combinations appear to be free of injury from tristeza. At the Estação Experimental de Citricultura de Limeira sweet orange (vars. Pera and Bahianinha) and grapefruit (var. Marsh seedless) on sweet orange, Rangpur lime, sweet lime, "cravo" tangerine, and rough lemon have shown no injury attributed to tristeza in plantings in which most of the trees of sweet orange and grapefruit on sour orange and bittersweet orange rootstocks are now dead or severely affected with the disease. Near Limeira an 8-acre grove of trees of sour orange on sweet orange rootstock has shown no symptoms of disease, although most of the trees of the reverse combination in this area have been killed. It is known also, as reported by Bitancourt (*6*), that when trees of sweet orange on sour orange rootstock are top-worked to lemon they become resistant to injury.

In tests with small grafted plants in pots the following combinations appear to be resistant to injury: Sweet orange on Duncan grapefruit, on Cleopatra mandarin, on Satsumelo 10–V–3, and on Sunshine tangelo; and Duncan grapefruit and Melancia shaddock on sour orange. Some of these combinations, however, need further testing before

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they can be definitely considered resistant. Plants of this experiment are undergoing further exposure to the disease in the field.

It seems probable that further information on varietal susceptibility may throw more light on the relation of tristeza to quick decline in California, bud-union decline in Australia, and the troubles in the Union of South Africa and Java, all of which have in common the ability to produce severe injury on trees of sweet orange on sour orange rootstock.

Marloth (*17*) found that trees of sweet orange and tangerine in Transvaal were injured when on sour orange rootstock, but that some degree of success was obtained with trees of grapefruit and tangelo on sour orange rootstock. The behavior of Sampson tangelo as a stock was found to be similar to that of sour orange. This evidence of susceptibility of Sampson tangelo as a stock, together with its resistance as a scion on sour orange in Brazil, indicates that the reaction of this variety of tangelo to the virus of tristeza may prove to be the same as that of sour orange, provided, of course, that the South African disease is caused by the same virus.

Oberholzer (*21*) stated that the commercial lemon, certain acid limes, and, to a certain degree, grapefruit react more or less in the same way as sour orange when used as rootstocks for sweet orange in the Union of South Africa.

In Java, Toxopeus (*33*) found "Japanese citron" (probably *Citrus nobilis* Lour. × *C. medica*) resistant when used as a stock for sweet orange, but when used as an interstock between a sweet orange scion and a sour orange rootstock it afforded no protection. When sweet orange, "Japanese citron," and sour orange were used as scions, interstocks, and stocks in all the possible combinations, injury occurred only in trees that had a sweet orange top and an interstock or a stock of sour orange. Terra (*32*) stated that, in Java, Cleopatra mandarin appears to make a satisfactory stock for sweet orange but that another tangerine, "Djeroek siem," was a failure as a stock for Pineapple orange. The "sour orange Peradeniya," which may or may not be a variety of sour orange, was resistant as a rootstock for sweet orange.

Trees of sweet orange on grapefruit rootstock have been reported (*30*) resistant to quick decline in California.

McAlpin, Parsai, Roberts, and Hope (*16*) listed the following combinations as susceptible to injury by bud-union decline in Australia: Sweet orange on sour orange, grapefruit, kumquat (*Fortunella* sp.), and lemon; grapefruit on sour orange and Eureka lemon; and mandarin on sour orange.

Whether the apparent differences in varietal reaction, such as the seeming differences in behavior of grapefruit as a stock for sweet orange in Brazil and in California, have any significance with respect to the causal agent in the two cases is not certain, and further observations are needed. From the limited evidence now available it may be expected that a considerable range of susceptibility to tristeza will be found among varieties of grapefruit and shaddock. This may be true also for tangerine, tangelo, and possibly other related types.

SYMPTOMLESS CARRIERS OF TRISTEZA VIRUS

Whether any recognizable effects are produced on seedling trees of any species or variety of citrus or citrus relatives by the tristeza virus

remains to be determined. A type of vein yellowing occurring alone or accompanied by some leaf drop on isolated twigs of many trees that otherwise appear normal, however, may be caused by the tristeza virus. The yellowing is of the type encountered on trees of sweet orange definitely affected with tristeza. It has been found in most striking form on grapefruit in new flushes of growth. Yellowing similar to that described on seedling trees has been observed occasionally also on trees of resistant scion-stock combinations. It would seem possible for this type of yellowing to be caused by an accumulation of materials above a stem zone in which there has been collapse of sieve tubes in an area temporarily susceptible to injury.

It is to be expected that all types that show tristeza when grafted on sour orange or other susceptible stocks will be virus carriers as seedlings or when grafted on resistant stocks after being infected.

At present, however, there seems to be no way of determining with certainty the presence of virus in such trees other than by transmission of virus from the suspected tree to trees of scion-stock combinations known to be susceptible to injury. In 1947 extensive attempts were made to transmit the virus from different species and varieties of citrus showing no symptoms of the disease to potted sweet orange plants on sour orange rootstock by infesting them with *Aphis citricidus*, by insertion of buds, and by cleft grafting twigs into stems of the plants to be inoculated. The results of the first of these tests are shown in table 5. They indicate rather definitely that the virus was present in certain citrus types in which no symptoms of the disease were recognized. The types that showed most convincing evidence of being virus carriers are rough lemon on its own roots, "cravo" tangerine on its own roots, the Pera variety of orange on Rangpur lime, and an unidentified citrus tree on its own roots. In this connection it is of interest that Fawcett and Wallace (*14*) have shown that the virus of quick decline is carried by trees of sweet orange that show no symptoms of the disease.

TABLE 5.—*Results of attempts to transmit tristeza virus to potted sweet orange plants on sour orange rootstock from various species and varieties of citrus exposed to infection but showing no symptoms*

Source of inoculum	Results with indicated type of inoculum					
	Aphis citricidus		Twigs		Buds	
	Plants infested	Plants infected	Plants grafted	Plants infected	Plants budded	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Rough lemon on own roots	0	----------	5	4	5	1
Grapefruit budded on grapefruit	0	----------	5	1	5	0
Eureka lemon budded on trifoliate orange	0	----------	5	0	5	0
"Cravo" tangerine on own roots	0	----------	5	5	5	1
Pera orange budded on Rangpur lime	10	1	5	5	5	4
Sweet orange (caipira) on own roots	5	0	5	1	5	1
Citron on own roots	5	0	5	0	5	0
Rangpur lime on own roots	0	----------	5	0	5	0
Sour orange on own roots	10	0	10	0	10	0
Trifoliate orange on own roots	0	----------	5	0	5	0
Unidentified citrus tree on own roots	5	5	0	----------	0	----------

RELATIVE SUSCEPTIBILITY OF SWEET AND SOUR ORANGES TO INFECTION

Since trees composed of sweet orange scions on sour orange rootstock are severely injured by tristeza and are therefore of greatest economic importance in relation to the disease, it was thought worth while to attempt to determine the relative susceptibility of plants of the constituent species to infection when growing on their own roots. For these tests potted plants of each species were divided into two lots when about 10 cm. tall. In the first test one lot of each species was inoculated three times by infestation with *Aphis citricidus* from diseased orange trees at intervals of 2 weeks and the other lot was not inoculated. The aphids were allowed to feed 48 to 72 hours at each infestation and then were destroyed.

Since no reliable symptoms of tristeza have been observed on either of these species of citrus when growing on its own roots, it was necessary to graft the sweet orange onto the sour orange plants in order to determine whether infection was produced by the aphids. Ten days after the third inoculation the following graft combinations of sweet and sour oranges were made: (1) Inoculated sweet orange on noninoculated sour orange; (2) noninoculated sweet orange on inoculated sour orange; and (3) noninoculated sweet orange on noninoculated sour orange.

In the second test small plants of sweet and sour oranges about 10 cm. tall were inoculated by infestation with *Aphis citricidus*. Two days after inoculation the aphids were killed and the three graft combinations listed for the first test, as well as inoculated sweet orange on inoculated sour orange, were made.

The results of these tests (table 6) show that small sour orange plants are susceptible to infection and that when used as stocks they transmitted the infection to noninoculated sweet orange scions. The results indicate strongly also that the sour orange plants were less susceptible to infection than the sweet orange plants. Since as a rule plants increase in resistance to virus diseases as they become larger, it seems probable that larger plants of sour orange may prove to be still more resistant.

TABLE 6.—*Results of grafting inoculated and noninoculated small seedling sweet orange plants on inoculated and noninoculated plants of sour orange*

[Inoculation by infestation with *Aphis citricidus*]

Test No.	Graft combination used	Plants tested	Plants showing disease
		Number	*Number*
1	Inoculated sweet orange on noninoculated sour orange	38	22
	Noninoculated sweet orange on inoculated sour orange	36	9
	Noninoculated sweet orange on noninoculated sour orange	20	0
2	Inoculated sweet orange on noninoculated sour orange	14	5
	Noninoculated sweet orange on inoculated sour orange	15	1
	Noninoculated sweet orange on noninoculated sour orange	18	0
	Inoculated sweet orange on inoculated sour orange	8	5

EVALUATION OF CONTROL MEASURES USED IN BRAZIL

Control measures for tristeza in Brazil have been of two kinds: (1) Those designed to save trees of susceptible scion-stock combinations from destruction and (2) those designed to avoid future losses in new plantings.

The measures that have been used to save trees of susceptible scion-stock combinations already planted consist of (1) inarching with resistant stocks; (2) mounding the soil to a level higher than the graft union to stimulate the production of roots from the scion; (3) top working with lemon; and (4) encouraging growth of sour orange shoots from the trunk or root system of the stock with the hope that these shoots will supply the carbohydrates necessary to replace those that are normally supplied by the sweet orange top.

The success of the method of control involving inarching depends largely on the size and kind of tree involved, on the time of infection of the treated tree in relation to time of inarching, and to some extent on environmental factors. In general, large trees, especially if inarched after they become diseased or shortly before, have not given encouraging results. However, after tristeza appeared in the grove at Fazenda São Sebastião, Caratuatatuba, 20,000 trees of Marsh seedless grapefruit budded on sour orange stock and varying in age from 6 to 12 years were inarched with resistant rootstocks with a high degree of success. Over 90 percent of the treated trees recovered or were maintained in a satisfactory condition. This grove is located near the coast, where high temperatures and humidity favor almost continuous growth.

In an orange grove belonging to the same firm, but located on the plateau, where the climate is drier and the temperatures lower, 27,000 trees of the variety Bahianinha and 5,000 trees of the variety Hamlin on sour orange rootstock, ranging in age from 6 to 12 years, were inarched with resistant stocks. Although about 70 percent of the young trees used in inarching became established, the inarched trees have not regained their former vigor and productivity.

In other orange groves in which inarching has been practiced some trees appear to have been benefited, but in areas where the disease is prevalent few inarched trees that have been maintained in a highly productive condition have been observed. It seems probable, however, that trees inarched well in advance of infection so that they would be largely on resistant roots before infection occurred could be saved.

With small trees there is evidence that control by inarching is more successful, and it is probable that most young groves could be saved from destruction by inarching with resistant varieties if inarching were started before invasion or in the early stages of invasion of the groves by the disease. In practice, the method has not been used extensively, probably largely because of the cost.

From the observations thus far trees of grapefruit appear to have given better results from the inarching method of control than have those of sweet orange. This may be due, in part at least, to a slower rate of decline of trees of grapefruit after infection, thus affording more

favorable conditions for the young trees used for inarching to become established.

The type of citrus used as rootstocks in inarching appears to be important in the success of the method. Observations made by A. J. Rodriegues [6] on three types of rootstocks indicated that Rangpur lime and sweet lime gave somewhat better results than sweet orange (caipira) in groves in which tristeza was present.

Mounding of the soil around the trunks of diseased or healthy trees of susceptible combinations to stimulate the growth of roots from the scion has not been tried extensively in Brazil, mainly because as a rule the trees are budded so high that large mounds are required to cover the base of the scion. Also some difficulty has been experienced in obtaining root development from the scion. The method may prove more effective where the trees are budded lower and where root rot is not a serious problem.

Top working susceptible orange trees with lemon has been very effective in controlling tristeza where the trees were not too large or too badly damaged when the top working was done. The practicability of this method of control depends, of course, largely on whether there is a market for the resulting lemon crop. Partly because of market limitations and demands this method of control has been used only to a limited extent.

In the Fazenda São Carlos near Palmeiras encouraging the growth of shoots from the trunk and root system of sour orange is being tested commercially on a large scale in a grove of 30,000 orange trees in which tristeza is just becoming established. Whether these shoots will supply the deficit in carbohydrates in the stock caused by the disease and thus prolong the productive life of the tree remains to be determined. Some trees have been observed with sour orange sprouts 3 to 5 years old growing from the trunk and from the root system, but the sweet orange tops in all cases have continued to decline.

Since a very high percentage of the orange trees of Brazil of scion-stock combinations susceptible to injury by tristeza have been destroyed or rendered of little value, the problem of control of the disease there is largely one of replanting with resistant trees and of choosing the most suitable resistant citrus types to replace sour orange as a rootstock. Brieger and Moreira (*8*) have presented extensive data on the performance, over a period of years, of 12 citrus types as rootstocks for the Marsh seedless variety of grapefruit and for the Pera and Bahianinha varieties of sweet orange. The types that have given best results in these tests are sweet orange (caipira and laranja lima), rough lemon, Rangpur lime, and sweet lime. These are the types now being used most extensively in Brazil as rootstocks for the commercial varieties of sweet orange.

It seems evident that it will be necessary to discard sour orange as a rootstock for varieties of sweet orange and perhaps for grapefruit and some varieties of tangerine and related types in all parts of the world where tristeza occurs unless types of sour orange more tolerant of the disease are obtained. Because of danger of spread of the disease to districts now free of infection, it may be advisable also to begin to

[6] Unpublished information communicated to A. S. Costa.

limit the use of sour orange as a rootstock in many districts where the disease has not been introduced.

Chiefly because of its resistance to certain types of phytophthora rots the loss of the sour orange will be felt keenly, especially in those areas that do not have open, well-drained soils. The spread of tristeza has emphasized the need for greater resistance to root rot and crown rot organisms in rootstocks that are resistant to tristeza. A very valuable contribution to the search for such resistance has been made already by Rossetti (*26*), who presented evidence that there is a considerable range of resistance to species of *Phytophthora* in seedlings of several varieties of sweet orange. Her work encourages the hope that in time rootstocks of sweet orange, and perhaps of other types, that have a higher degree of resistance to root rot organisms may be found. The investigations stimulated by the ravages of tristeza may be expected to add materially to the knowledge of the value of many types of citrus for use as rootstocks.

DISCUSSION

Tristeza of citrus is characterized largely by failure to produce primary symptoms on the foliage of any known host plant and by the production of severe effects only on trees of certain scion-stock combinations, the best known of which is sweet orange on sour orange rootstock. Neither of the component species of the latter combination is known to be susceptible to injury when on its own roots, and no injury is evident on trees composed of sour orange tops on sweet orange roots. Scion-stock relations in some respects similar to this have been reported by Rawlins and Parker (*24*) for buckskin disease of cherry, by Raleigh (*23*) for potato latent mosaic, and by Gardner, Marth, and Magness (*15*) for a disease of apple which follows the grafting of certain apple scions on Spy 227 rootstock.

Those symptoms so far described for tristeza are secondary and are of a type produced readily by a partial or complete girdle. Evidence indicates, in fact, that a form of girdling is responsible for the known symptoms of the disease. Schneider, Bitancourt, and Rossetti (*29*) have shown that in trees affected by tristeza sieve tubes and companion cells below the union of scion and stock become necrotic. Injury of this type would be expected to lead to an excessive accumulation of metabolites in the top of the tree and to a food deficiency in the root system. Thus, it might be expected that the first symptoms of the disease would be associated with the accumulation of excessive amounts of carbohydrates and other products in the tops of the trees and that the later symptoms might be influenced to a greater degree by injury to the root system resulting from deficiency of food materials.

The sequence of symptoms produced in young trees inoculated with tristeza virus in the field followed this expected pattern. Leaves of infected trees 4 to 6 months after inoculation took on a dull appearance, often with a tendency toward vein yellowing. This first noticeable injury did not appear to be due to root deterioration, since no evidence of starch depletion or root decay was detected in tests made shortly after symptoms began to appear. Symptoms somewhat similar, but characterized by more vein yellowing, were produced in about the

same length of time on check trees on limbs around which a wire had been wrapped.

Also similar secondary symptoms of vein yellowing and chlorosis were observed on potted orange plants heavily infested with cottony-cushion scale (*Icerya purchasi* Mask.). This yellowing is thought to have been associated with injury to the phloem by insect-produced toxins.

The peculiar scion-stock relation in the production of symptoms of tristeza is of special interest. It seems probable that for the production of injury in the species and varieties of citrus so far observed (1) the scion must be of a species or variety which produces relatively large quantities of virus but which is tolerant of the virus and of products resulting from its presence and (2) the rootstock or the interstock must be of a species or variety which is capable of producing relatively low concentrations of the virus and which is highly sensitive to the virus or to products resulting from its presence.

Plants of a variety or species capable of producing high concentrations of the virus or of virus toxin and having phloem sensitive to injury by it would be expected to show symptoms on their own roots and would be valuable as test plants for use in the study of the disease. As yet no plant of this type has been discovered.

Information available thus far seems to indicate that the sweet orange is relatively susceptible to infection and that it may be capable of supporting relatively high concentrations of virus, but that the sour orange is less susceptible to infection and may have lower concentrations of virus. It seems probable, therefore, that infection of a tree composed of a sweet orange scion and a sour orange rootstock would be followed by multiplication of the virus in the sweet orange tissue and by the production of relatively high concentrations of virus in the top. Since it has been demonstrated that viruses that enter the phloem move rapidly in the direction of translocation of elaborated food products, it seems probable that the toxic principle, whether it be a virus or a toxin resulting from the presence of the virus, would move rapidly and in considerable quantity from the scion into the stock and that the concentration of injurious material in the sour orange, particularly immediately below the union between scion and stock, would be much higher than could be reached as a result of virus increase in a sour orange tree.

It is also conceivable that virus multiplication in a sour orange rootstock might be increased greatly by a sweet orange top if the assumption is made that virus increase takes place at the expense of materials carried in the phloem and that materials are carried in the phloem as a result of mass movement of the liquid phloem content. In this case it would be expected that virus precursors built up in the sweet orange would move from the sweet orange to the sour orange and that the phloem content of the sour orange stock immediately below the union with the scion would be more characteristic of the phloem content of sweet orange than of sour orange. Thus by virtue of these materials increase might occur, and the virus or virus toxin might reach concentrations sufficiently high to cause collapse of sieve tubes.

There is evidence in the case of at least one virus to support the idea that materials can move from a susceptible virus-infected scion and

produce symptoms of disease on a resistant stock. When a scion of Turkish tobacco infected with a virus which is believed to be a mutant of the sugar-beet curly top virus and which produces marked yellowing of veins of leaves of Turkish tobacco is grafted onto a plant of *Nicotiana glauca*, the first leaves of the new shoots from the stock have yellow veins. This condition persists as long as the shoots from the stock are in a position to receive food material from the scion, but when they grow and presumably become independent of the scion for their food supply they no longer produce leaves with yellow veins. For all practical purposes *N. glauca* is immune from this virus, for when the scion is removed the stock no longer produces leaves with yellow veins and soon the virus is lost.

On the basis of these concepts theories may be advanced to explain certain anomalies such as injury in trees having an intergraft of sour orange between a sweet orange top and a resistant root and the resistance of trees with an intergraft of sweet orange between a lemon top and a sour orange rootstock. In the first instance the virus would move from a susceptible sweet orange, possibly highly charged with virus, into sour orange tissue and collapse of sieve tubes would be expected in the sour orange regardless of the type of tissue below the sour orange interstock. In the second instance, although movement into the sour orange stock would be from sweet orange, the materials moving from the sweet orange into the sour orange probably would be largely those manufactured by lemon. Since it seems probable that lemon is a relatively unfavorable medium for virus increase, only low concentrations of toxic material at most would move from the lemon through the sweet orange interstock into the sour orange stock. These concentrations might well be too low to cause serious injury to the phloem.

The reason for the failure of tristeza virus to produce appreciable injury on any species or variety of citrus growing on its own roots is of considerable interest from both a scientific and a practical viewpoint. The most obvious explanation of this curious fact would appear to be that no species or variety known at present is able to produce sufficient concentrations of virus or of virus toxin within its own tissues to cause appreciable injury to its own phloem. On the basis of this theory it seems probable that there may eventually be found citrus species and varieties on their own roots that show five more or less well defined relations to the virus, only three of which have as yet been encountered with any degree of certainty. These relations may be indicated as follows:

(1) The plant may permit little or no virus increase but possess phloem tissue very susceptible to injury by the virus or toxin. Sour orange, Sampson tangelo, and some true lemons show evidence of such a relation.

(2) The plant may permit production of moderate concentrations of virus or toxin and possess phloem tissue only moderately susceptible to injury. Some varieties of grapefruit and shaddock may have this relation.

(3) The plant may permit the production of high concentrations of virus and possess phloem tissue highly resistant to injury. Sweet

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orange, and perhaps Rangpur lime, rough lemon, and some varieties of tangelo and mandarin, may have this relation.

(4) The plant may permit the production of little or no virus and possess phloem tissue that is tolerant of high concentrations of virus or toxin resulting from the presence of virus. Varieties showing this relation are not known, but possibly some varieties of lemons or other resistant types have such a relation. A variety of this kind would be expected to be resistant both as a stock and as a scion for other citrus varieties.

(5) The plant may permit the production of relatively high concentrations of virus and possess phloem very sensitive to injury. Such a plant, of course, should show symptoms as a seedling and would be of value in experimental work.

On the basis of these concepts it would be expected that any given scion-stock combination will prove to be resistant to injury by tristeza if the stock is of a variety that on its own roots is capable of producing a virus concentration as high as that normally produced by the scion variety on its own roots or higher. Also, it may be expected that any given scion-stock combination will prove to be susceptible if the stock is of a variety that on its own roots produces a lower concentration of virus or toxin than that produced by the scion variety unless the stock variety falls in class 4. With many varietal combinations it is possible that the degree of injury to the tree may be to some degree proportional to the difference in these respective concentrations in stock and scion varieties.

These postulated relations provide a basis for a logical explanation of the seemingly contradictory observations that grapefruit is susceptible to injury as a scion when it is on a sour orange rootstock and apparently susceptible to injury also as a stock when the scion variety is sweet orange, whereas it is less susceptible to injury when both stock and scion are grapefruit. Grapefruit trees on sour orange stocks are injured less than sweet orange trees on sour orange stocks, indicating that grapefruit produces a smaller amount of toxic materials than sweet orange. Also trees of sweet orange on grapefruit stocks are injured less than trees of sweet orange on sour orange stocks, indicating that grapefruit is less susceptible than sour orange as a stock. Thus a lower concentration of virus or toxin in grapefruit as a scion together with greater resistance of grapefruit as a stock may result in resistance to injury in a tree composed of a grapefruit top on a grapefruit root, even though grapefruit may be susceptible to injury both as a stock and as a scion in certain other scion-stock combinations.

At the Instituto Agronômico at Campinas, Brazil, the concepts listed have been the basis for planning some of the tests having for their objective the determination of relative tristeza-resistance values of citrus species and varieties both as scions and as stocks. In addition to the direct testing of numerous types of citrus as rootstocks for sweet orange, grapefruit, and other types, an indirect test in which numerous citrus types are grafted onto sour orange is being made. It is expected that, in general, the degree of injury shown by these types on sour orange will be a relative measure of the concentration of virus or virus toxin that the scion variety is able to tolerate without injury. Scions that show severe injury on sour orange rootstock may prove to

be resistant as rootstocks for varieties that show less injury as scions on sour orange rootstock. Thus it may be that varieties capable of producing high concentrations of virus will prove to be among the best rootstocks from the standpoint of resistance to injury by tristeza.

The relation of tristeza in Brazil to similar disorders in other places, notably the Union of South Africa, Argentina, Uruguay, Java, Australia, and southern California, has been the subject of considerable speculation. However, in some cases more evidence on which to base definite conclusions is needed. Tristeza in Brazil appears to be identical with podredumbre de las raicillas in other South American countries. There are marked similarities also between tristeza and the disorders which occur on sweet orange varieties on sour orange rootstock in the Union of South Africa and Java. Olmo and Moreira (*22*) emphasized the similarities between tristeza in South America and quick decline in California. Schneider (*28*) pointed out that almost identical types of phloem necrosis are found in trees infected with tristeza and with quick decline; and Meneghini (*19*) emphasized similarities in starch depletion in the sour orange rootstock.

Despite these striking similarities certain variations in the effects produced in different parts of the world suggest that the diseases are not identical in all instances. Some of the observed differences, such as the reported resistance of trees of sweet orange on grapefruit rootstock to quick decline and the failure of such trees to develop normally in certain districts in which tristeza is present, may be due to varietal or other differences. Variation in certain symptoms, for example, may be related to soil and climatic conditions. It is not clear, however, that all of the observed variations are the result of varietal and environmental influences.

Toxopeus (*33*) reported that in Java buds from sweet orange died in 8 to 12 months after being placed in sour orange stocks, and Oberholzer (*21*) stated that in the Union of South Africa young trees formed by budding Valencia orange into susceptible rootstocks began to exhibit extensive root decay, chlorosis, and general decline at a very early age, usually 1 to 3 months after budding. Such rapid decline or killing has not been noted in Brazil after the budding of either diseased or healthy sweet orange buds into sour orange rootstock. In experimental tests buds from sweet orange trees affected with tristeza budded into healthy sour orange rootstocks developed more or less normally at first and after 12 months they showed a reasonably high degree of vigor. In nursery trees inoculated with the tristeza virus in Brazil by insertion of buds from diseased trees, the incubation period of the disease, as measured by leaf discoloration, was as short as 4 months, in comparison with about 15 months observed by Fawcett and Wallace (*13*) for quick decline in California. Also in trees affected with tristeza (*2, 7, 25*) and with quick decline (*11*) there appear to be differences in the amount of starch found in the bark of sweet and sour oranges in the vicinity of the graft union and in that found in the roots of the sour orange rootstock in certain stages of development of the disease.

If these differences prove not to be due to varietal and environmental influences, they indicate that tristeza, as it is known in Brazil, differs in some respects from similar disorders in Java, California, the

Union of South Africa, and perhaps Australia. If these disorders are not caused by the same virus, then it must be assumed on the basis of information now available that they are caused either by closely related viruses or by virus strains that vary in virulence and possibly in other characteristics. The latter alternative seems the more tenable at present.

If tristeza originated in the Union of South Africa as has been suggested, all the strains of the causal virus would be expected to occur there provided no mutation has occurred in areas more recently invaded. If virus was carried from southern Africa to other parts of the world, as seems likely, it would be expected that not all of the strains would necessarily be introduced into any one place at a given time. The characteristics of the disease produced in the different countries would be expected to vary, therefore, according to the strain or strains of the virus introduced.

If strains of a single virus or even related viruses cause the various disorders and if these strains or related viruses differ in their ability to attack different species and varieties of plants, as is known to be the case with certain other virus diseases, it seems very important to obtain as soon as possible information on the reaction of a wide range of varieties and species of citrus in all parts of the world where the disorders occur. This is true especially of varieties of grapefruit, mandarin, lemon, tangelo, tangerine, and any other types that may be considered for use as rootstocks to replace sour orange.

SUMMARY

Tristeza of citrus, first observed in Argentina in 1930 or 1931 and in Brazil about 1937, now occurs in most of the commercial citrus districts of Argentina, Brazil, and Uruguay. The same or similar disorders, all characterized by ability to cause serious damage to varieties of sweet orange on sour orange rootstock, have been reported from the Union of South Africa, Java, southern California, and Australia.

In experimental work at Campinas, Brazil, transmission of tristeza was not obtained by means of juice from diseased sweet orange plants. Of 91 sweet orange plants inoculated by establishing on them dodder (*Cuscuta* spp.) that had grown on diseased plants, 1 plant inoculated by means of *C. subinclusa* became infected and 1 inoculated by means of *Cuscuta* sp. showed symptoms resembling tristeza, indicating that the disease may be transmissible to a low percentage of plants by this means.

The disease was transmitted to 10 varieties of sweet orange on sour orange rootstock growing under field conditions by the insertion of buds from diseased trees and to sweet orange plants on sour orange rootstock in the greenhouse by the insertion of buds and twigs from diseased trees.

The causal virus was transmitted to small plants under greenhouse conditions by infesting them with the oriental citrus aphid (*Aphis citricidus*) from diseased plants, confirming the transmission results of Meneghini with this insect. A low percentage of plants infested with single aphids became infected, but high percentages of infection were obtained only with large numbers of insects. In limited tests a period of starvation prior to feeding on diseased plants appeared not

to increase the ability of the insects to transmit the virus. Transmission was obtained with aphids only after they had fed 60 minutes or longer on diseased plants.

No transmission was obtained by means of any of 10 other species of insects, including 5 of aphids, 4 of leafhoppers, and 1 of white fly, after they had fed on diseased sweet orange trees.

A number of species and varieties of annual plants inoculated with juice from infected sweet orange trees by establishment of dodder from such trees and by infestation with *Aphis citricidus* developed no symptoms that indicated transmission of the virus.

In tests involving more than 1,400 plants the disease was not transmitted through the seeds of sweet or sour orange.

No symptoms known to be caused by tristeza have been observed on any variety of citrus or citrus relative on its own roots, although it is thought possible that certain types of vein yellowing observed occasionally on isolated twigs of trees of grapefruit and many other types of citrus may be caused by the disease. The scion-stock combinations that have been observed to be injured are the following: Sweet orange, Marsh seedless grapefruit, Mexeriqueira tangerine, mandarin, Thornton tangelo, and Galacian lemon on sour orange rootstock, and probably sweet orange on grapefruit rootstock. Results from inoculation of small plants by means of *Aphis citricidus* have indicated that the following scion-stock combinations may be susceptible to injury under greenhouse conditions: Sweet orange on Viçosa grapefruit and Doce and Melancia shaddocks; and Viçosa grapefruit, Doce shaddock, Cleopatra mandarin, Satsumelo 10–V–3, and Sunshine tangelo on sour orange rootstock.

In tests in which small seedlings were inoculated by means of *Aphis citricidus*, sweet orange seedlings appeared to be more susceptible to infection than sour orange seedlings.

The virus was transmitted to small sweet orange plants on sour orange rootstock from trees of the following scion-stock combinations that showed no evidence of disease: Pera variety of sweet orange on Rangpur lime, rough lemon on its own roots, "cravo" tangerine on its own roots, and an unidentified citrus tree on its own roots.

Methods of control that have been used in citrus plantings in Brazil are (1) inarching with resistant stocks, (2) mounding of the soil around the base of the trees to induce the production of a root system from the scion variety, (3) top working with lemon, and (4) encouraging the growth of sour orange shoots from the stock with the hope that they will supply to the root system the necessary food materials to prolong the productive life of the trees. For various reasons none of these has proved satisfactory for general use. Since a high percentage of the bearing trees of Brazil were on sour orange rootstock and most of these have been killed or rendered valueless, control measures in the future will consist chiefly in making new plantings with trees of scion-stock combinations that are resistant to injury. Thus far the most satisfactory rootstocks have proved to be sweet orange, rough lemon, Rangpur lime, and sweet lime.

LITERATURE CITED

(1) Bennett, C. W., and Costa, A. S.
1948. A PRELIMINARY REPORT OF WORK AT CAMPINAS, BRAZIL, ON TRISTEZA DISEASE OF CITRUS. Fla. State Hort. Soc. Proc. (1947) 60: 11–16.

(2) Bertelli, J. C.
1947. PRIMER AGREGADO AL ESTUDIO DE LA ETIOLOGÍA DE LA PODREDUMBRE DE LA[S] RAICILLA[S] O "TRISTEZA" DE LOS CITRUS. Uruguay Min. de Ganad. y Agr., Dir. de Agron., Pub. 91, 16 pp., illus.

(3) ——— and Bertelli, L., K. de.
1944. PODREDUMBRE DE LAS RAICILLAS DE LOS CITROS. Uruguay Min. de Ganad. y Agr., Lab. de Fisiol. y Patol. Veg., [Pub.] 71, 23 pp., illus.

(4) Bitancourt, A. A.
1940. A DOENÇA DOS CITRUS NO VALE DO PARAÍBA. Biológico 6: 268–269.

(5) ———
1940–41. A PODRIDÃO DAS RADICELAS DOS CITRUS NA PROVINCIA DE CORRIENTES, ARGENTINA. Biológico 6: [285]–288, 356–364, illus., 1940; 7: 62–69, 1941.

(6) ———
1943. RECOMENDAÇÕES PARA COMBATER E MINORAR OS ESTRAGOS DA "PODRIDÃO DAS RADICELAS" DOS CITRUS. Biológico 9: [41]–42.

(7) ———
1944. UM TESTE PARA A IDENTIFICAÇÃO PRECOCE DA TRISTEZA DOS CITRUS. Biológico 10: 169–175, illus. [English summary, p. 175.]

(8) Brieger, F. G., and Moreira, S.
1945. EXPERIÊNCIAS DE CAVALOS PARA CITRUS II. Bragantia 5: [597]–658, illus.

(9) Carrera, C.
1933. INFORME PRELIMINAR SOBRE UNA ENFERMEDAD NUEVA COMPROBADA EN LOS CITRUS DE BELLA VISTA (CORRIENTES). Argentina Min. de Agr. Bol. Mens. 34: [275]–280, illus.

(10) ———
1935. INFORME DE LAS OBSERVACIONES Y EXPERIMENTACIONES EFECTUADAS SOBRE UNA NUEVA ENFERMEDAD APARECIDA EN LOS CITRUS DE BELLA VISTA (CORRIENTES). Argentina Min. de Agr. Bol. 37: [15]–[37], illus.

(11) Fawcett, H. S.
1945. A STARCH TEST FOR QUICK DECLINE. Calif. Citrog. 30: 122.

(12) ———
1946. A PROGRESS REPORT ON QUICK DECLINE STUDIES: INTRODUCTION (PART I); STARCH RELATIONSHIPS (PART II). Calif. Citrog. 31: 198.

(13) ——— and Wallace, J. M.
1946. EVIDENCE OF THE VIRUS NATURE OF CITRUS QUICK DECLINE. Calif. Citrog. 32: 50, 88–89, illus. (Also in Citrus Leaves 26 (12): 9–10, illus. 1946.)

(14) ——— and Wallace, J. M.
1948. SWEET-ROOT ORANGE TREES, SYMPTOMLESS CARRIERS OF QUICK DECLINE VIRUS. Citrus Leaves 28 (3): 6.

(15) Gardner, F. E., Marth, P. C., and Magness, J. R.
1946. LETHAL EFFECTS OF CERTAIN APPLE SCIONS ON SPY 227 STOCK. Amer. Soc. Hort. Sci. Proc. 48: 195–199.

(16) McAlpin, D. M., Parsai, P. S., Roberts, R., and Hope, R. H.
1948. "BUD-UNION DECLINE" DISEASE IN CITRUS TREES. Victoria Dept. Agr. Jour. 46: 25–31, illus.

(17) Marloth, R. H.
1938. THE CITRUS ROOTSTOCK PROBLEM. CITRUS-TREE PROPAGATION. Farming in So. Africa 13: 226–231, illus.

(18) Meneghini, M.
1946. SÔBRE A NATUREZA E TRANSMISSIBILIDADE DA DOENÇA "TRISTEZA" DOS CITRUS. Biológico 12: 285–287.

(19) MENEGHINI, M.
1947. REAÇÃO DE AMIDO NAS ENXERTIAS DE "SEEDLINGS" DE LARANJEIRA UTILIZADAS EM EXPERIÊNCIAS DE TRANSMISSÃO DA "TRISTEZA." Biológico 13: 91–92, illus.
(20) MOREIRA, S.
1942. OBSERVAÇÕES SOBRE A "TRISTEZA" DOS CITRUS, OU "PODRIDÃO DAS RADICELAS." Biológico 8: [269]–272.
(21) OBERHOLZER, P. C. J.
1947. THE BITTER-SEVILLE ROOTSTOCK PROBLEM. Farming in So. Africa 22 (255): 489–495, illus.
(22) OLMO, H. P., and MOREIRA, S.
1947. "QUICK DECLINE DISEASE" AND TRISTEZA. Calif. Citrog. 32: 138, 175–176, illus. (Also in Citrus Indus. 28 (4): 14–16, 18. 1947.)
(23) RALEIGH, W. P.
1936. AN ABNORMAL GRAFT REACTION IN POTATO RESULTING FROM A VIRUS INFECTION OF A SCION ON A RESISTANT STOCK. (Phytopath. note) Phytopathology 26: 795.
(24) RAWLINS, T. E., and PARKER, K. G.
1934. INFLUENCE OF ROOTSTOCKS ON THE SUSCEPTIBILITY OF SWEET CHERRY TO THE BUCKSKIN DISEASE. Phytopathology 24: 1029–1031.
(25) ROSSETTI, V.
1945. O TESTE DO IÔDO NA IDENTIFICAÇÃO DA "TRISTEZA" DOS CITRUS. Biológico 11: 13–21, illus.
(26) ———
1947. PORTA-ENXERTOS DE CITRUS RESISTENTES À "GOMOSE" DE PHYTOPHTHORA E À "TRISTEZA." Biológico 13: 89–90.
(27) SCHNEIDER, H.
1946. A PROGRESS REPORT ON QUICK DECLINE STUDIES: HISTOLOGICAL STUDIES (PART III). Calif. Citrog. 31: 198–199, illus.
(28) ———
1947. QUICK DECLINE AND TRISTEZA SIMILARITIES. Citrus Leaves 27 (8): 10–11, illus.
(29) ——— BITANCOURT, A. A., and ROSSETTI, V.
1947. SIMILARITIES IN THE PATHOLOGICAL ANATOMY OF QUICK-DECLINE- AND TRISTEZA-DISEASED ORANGE TREES. (Abstract) Phytopathology 37: 845–846.
(30) SMOYER, K. M.
1947. A PROGRAM FOR QUICK DECLINE. Pacific Rural Press and Calif. Farmer (South. Ed.) 154: 451. (Also in Citrus Leaves 27 (11): 10–11. 1947.)
(31) TAKAHASHI, R.
1931. APHIDIDAE OF FORMOSA.—VI. Formosa Govt. Res. Inst. Dept. Agr. Rpt. 53, 127 pp., illus.
(32) TERRA, G. J. A.
1947. JAVA'S CITRUS ROOTSTOCK DECLINE PROGRAM. Citrus Leaves 27 (6): 18, 20, 22.
(33) TOXOPEUS, H. J.
1937. STOCK-SCION INCOMPATIBILITY IN CITRUS AND ITS CAUSE. Jour. Pomol. and Hort. Sci. 14: 360–364, illus.
(34) WALLACE, J. M., and FAWCETT, H. S.
1947. QUICK DECLINE OF ORANGE TREES—A VIRUS DISEASE. Science 105: 315–316.
(35) WEBBER, H. J.
1943. THE "TRISTEZA" DISEASE OF SOUR-ORANGE ROOTSTOCK. Amer. Soc. Hort. Sci. Proc. 43: 160–168. (Portuguese translation by A. A. Bitancourt: Biológico 9: [345]–355. 1943.)

INHERITANCE OF A GENE FOR NEAR-WILT RESISTANCE IN THE GARDEN PEA[1]

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INTRODUCTION

Fusarium wilt of garden pea (*Pisum sativum* L.) was first recorded by Jones and Linford (*2*) [3] in 1925. In a later paper Linford (*3*) described the disease and the causal organism as *Fusarium orthoceras* (App. and Wr.) var. *pisi* Linford. He pointed out that some varieties are very resistant to the disease. Wade (*10*) studied the inheritance of the resistance to wilt and concluded that it was controlled by a single dominant gene. Walker (*11*) found that resistance to the disease was present in many varieties, absent in others, and variable in different seed lots of a few varieties. He pointed out that development of resistant varieties would involve only pure-line selection in some varieties while others would require selection from the progeny of hybrids with a resistant parent. Work along both these lines by breeders in the following years provided a range of wilt-resistant varieties for canning, many of which were adopted widely by the trade.

When wilt-resistant varieties came into general use, a closely similar disease was discovered (*5, 13*) which had been masked apparently by the more rapidly developing wilt disease. Snyder and Walker (*7*) described the new disease in detail in 1935 and called it near-wilt because of the similarity of its symptoms to those of wilt. They designated the causal organism as *Fusarium oxysporum* Schl. f. 8 Snyder. The wilt and near-wilt organisms were designated later by Snyder and Hansen (*6*) as *Fusarium oxysporum* f. *pisi* (Linford) race 1 Snyder and Hansen and *F. Oxysporum* f. *pisi* (Snyder) race 2 Snyder and Hansen, respectively.

Most varieties resistant to wilt were found to be susceptible to near-wilt (*8*). In a few, e. g., Rogers K and Horal, a moderate degree of resistance was exhibited but was not readily fixed by inbreeding. It appeared to be polygenic in inheritance and markedly influenced by

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[3] Numbers in parentheses refer to Literature Cited, p. 249.

environment. One line of breeding material was discovered which appeared to have practically complete field resistance to near-wilt. A selection of this line was increased and eventually introduced as a near-wilt resistant variety under the name Delwiche Commando in 1945 (*1*). In order to study critically the nature of inheritance of this newly discovered type of resistance to near-wilt and to transfer it to other varieties, crosses were begun in 1942. This paper is an account of the inheritance of resistance to near-wilt, a preliminary report of which has already been made (*12*).

METHODS AND MATERIALS

Field tests were made on soil naturally infested with the near-wilt organism, except in 1947. From 1938 to 1942, inclusive, a near-wilt plot near DeForest, Wis., was used. From 1943 to 1946, inclusive, trials were conducted near Waupun, Wis. This plot was in a field mapped for severity of infestation in 1941 when most plants in a commercial planting became severely diseased. In 1943, plantings were made on May 13 in rows 4 feet apart at about 8 seeds per foot of row. In each row, 6-foot strips of Wisconsin Perfection, a variety resistant to wilt but susceptible to near-wilt, were alternated with 18-foot strips devoted to other varieties, strains, or hybrids. Ordinary woven-wire fence was erected along the row to support the plants. Cultural practices commonly used to produce a canning crop were employed. At appropriate times, usually between the early-pod and canning stages, the plants were classified as resistant or susceptible to near-wilt. When conditions were favorable for disease development, as was the case in 1943, no trouble was encountered in making determinations.

Under conditions favorable to disease development susceptible plants, with the exception of a few scattered escapes, yellowed, wilted, and died rapidly. In the field, the xylem was generally brick red in color by the time the plants wilted. Under less favorable conditions the cause of the death of a plant was often questionable, and under some conditions susceptible plants showed no signs of disease at any time.

Plantings in 1944 were made on May 15 and 16 in the same manner as in 1943. No fence was used. Check plantings were spaced as in 1943 except where the hybrid did not involve Wisconsin Perfection; then the susceptible parent was alternated with the latter variety. Dates of planting and the methods of culture in subsequent years were similar to those of 1944.

In the greenhouse, 12 seeds were planted in each of 8 rows in sterilized quartz sand contained in metal pans 3 x 12 x 18 inches in dimension. The plants were watered as required with a balanced nutrient solution. The pans were placed in Wisconsin soil-temperature-control tanks held at 21° ±2° C. The air temperature was more variable than the sand temperature, but it averaged about 22° C. At 7 to 10 days after seed was sown the plants were removed and the taproots were clipped to a uniform length of 1 inch while the whole root system was immersed in a heavy suspension of spores and mycelial fragments of the near-wilt fungus. The plants were replaced in the

pans after inoculation. Inoculum was grown on a modification of Czapek's solution [4] in 6-liter flasks placed on a mechanical shaker.

Disease ratings were made approximately 3 weeks after inoculation. The segregating lines were compared with resistant Delwiche Commando and susceptible Wisconsin Perfection in each pan.

EXPERIMENTAL RESULTS

RESISTANT PARENT

In the course of screening several hundred breeding progenies for resistance to near-wilt it was noted that one line (738–49) remained practically free from near-wilt on the DeForest plot, while such wilt-resistant varieties as Wisconsin Perfection succumbed completely. When the former line was increased it maintained the same high degree of resistance and it was eventually released as Delwiche Commando. Line 738–49 was derived from a cross between Pride, a near-wilt susceptible variety, and a breeding line, 732–66–1. The latter was an F_3, progeny from a cross between two lines of Admiral. A mass increase of 732–66–1, tested in 1938, yielded 75 percent resistant and 25 percent susceptible plants. Of seven single plants selected from the last-mentioned resistant group tested on near-wilt soil, the progeny of one contained 100 percent susceptible plants, and those of five segregated at ratios closely approximating 3 resistant to 1 susceptible. Three single-plant selections from the last-mentioned segregating lines segregated in the same ratio in 1940. It was apparent, therefore, that the near-wilt resistance in 738–49 had been derived from the 732–66–1 parent. Furthermore, there was a strong indication that the resistant character in this line was controlled by a single dominant gene.

FIELD TRIALS WITH HYBRID PROGENIES

In order to transfer this type of resistance to other canning types and to determine its mode of inheritance, 738–49 was crossed with numerous other varieties. The initial study of inheritance reported herein was concerned primarily with crosses between 738–49 and four wilt-resistant, near-wilt susceptible varieties, Wisconsin Perfection, Perfected Wales, Merit, and Penin. The last two varieties had been derived from a cross between Horal and Prizewinner. They each contained a moderate amount of resistance to near-wilt characteristic of that which, as already mentioned, was noted earlier in Horal. This latter type of resistance was not linked with the type of resistance in 738–49, but, as will be noted later, its expression in hybrid material sometimes obscured that of the resistant character from 738–49.

In the fall of 1942, five sister lines of 738–49 designated as 738–49/I, II, III, IV, and V were crossed with Wisconsin Perfection, Perfected Wales, and Merit and 738–49/I was crossed with Penin. The F_1 generation of these crosses was grown in the greenhouse in the spring of 1943. From the resulting seed, F_2 progenies were grown on the Waupun plot. The near-wilt disease appeared during the first week in July. On July 16, all the plants in Wisconsin Perfection controls were wilted or dead except for a few in one corner of the plot. Most.

[4] KNO_3, 3.0 gm.; KH_2PO_4, 1.0 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; KCl, 0.5 gm.; $FeSO_4$, trace; dextrose, 30 gm.; water, 1,000 ml.

of the escapes had definite symptoms of near-wilt by July 20 although many of them were not dead. Progeny tests with seed from the occasional healthy survivors yielded plants 100 percent of which became diseased when tested in 1944, showing that the healthy plants in the controls were escapes. Four counts of diseased plants were made, the wilted ones being discarded at each reading. The results are presented in table 1.

TABLE 1.—*Occurrence of near-wilt in F_2 progenies from crosses between resistant and susceptible lines*

Cross No.	Parents	Plants wilted on date indicated				Total plants wilted	Total plants healthy	Chi-square for goodness of-fit to 3:1 ratio
		July 9	July 12	July 16	July 20			
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	
C 125	Perfected Wales × 738-49/I	5	5	36	29	75	197	0.83
C 129	Perfected Wales × 738-49/II	2	4	7	0	13	47	.20
C 132	Perfected Wales × 738-49/III	7	1	10	0	18	33	2.36
C 135	Perfected Wales × 738-49/IV	3	2	11	0	16	30	1.86
C 138	Perfected Wales × 738-49/V	1	0	18	2	21	58	.04
C 126	Merit × 738-49/I	6	0	5	10	21	94	2.44
C 130	Merit × 738-49/II	4	1	9	0	14	25	1.92
C 133	Merit × 738-49/III	12	0	4	3	19	28	5.17*
C 136	Merit × 738-49/IV	0	0	8	1	9	28	(1)
C 139	Merit × 738-49/V	0	0	3	2	5	27	1.04
C 127	Penin×738-49/I	11	1	69	3	84	150	14.25**
C 128	Wisconsin Perfection × 738-49/I	23	7	49	5	84	234	.27
C 131	Wisconsin Perfection × 738-49/II	1	2	10	0	13	45	.09
C 134	Wisconsin Perfection × 738-49/III	7	0	8	0	15	43	(1)
C 137	Wisconsin Perfection × 738-49/IV	10	4	7	0	21	41	2.15
C 140	Wisconsin Perfection × 738-49/V	15	4	4	0	23	59	.26
	738-49/I	0	0	4	1	5	[2] 500	
	738-49/II	0	0	16	0	16	[2] 500	
	738-49/III	0	0	7	1	8	[2] 500	

*Significant, 5-percent level; **significant, 1-percent level.
[1] Value for Chi-square less than 0.01.
[2] Approximate number.

Three sister lines of 738–49, one of which was later released as Delwiche Commando, were grown along with the F_2 progenies. The 738–49 lines were highly resistant to the disease except for 1 to 3 percent of the plants which wilted and died with symptoms that resembled but were not entirely typical of those of near-wilt. One of of the 738–49 lines is shown in figure 1, adjacent to the susceptible Wisconsin Perfection control. Fourteen of the 16 F_2 progenies showed a good fit to the ratio of 3 healthy plants to 1 diseased. When the crosses were made, the seeds from all pods that set in a given cross were bulked. An occasional pod may have resulted from self-pollination of the susceptible parent. This may account for the poor fit in C127, which was due to an excess over the expected number of susceptible plants.

Selections were made from the surviving F_2 plants in 1943. Some of these were planted in the greenhouse during the following winter. The resulting F_4 generations and the remnants of F_3 progenies were planted in the disease plot near Waupun in 1944. Near-wilt did not appear in the plot until the second week of July. Disease notes were taken on July 17, 20, and 24. The reactions of the F_3 familes in 1944

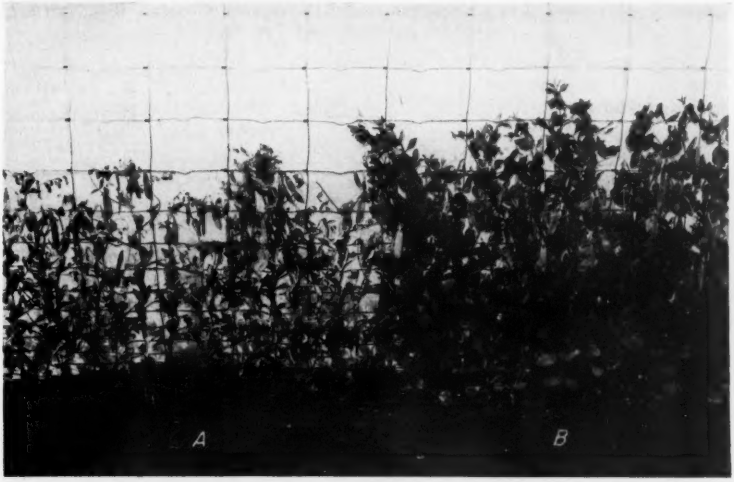

FIGURE 1.—Peas grown on soil naturally infested with *Fusarium oxysporum* f. *pisi* race 2 near Waupun, Wis., in 1943: *A*, Wisconsin Perfection, susceptible; *B*, 738–49/I, resistant, a sister line of which was increased and released as a new near-wilt resistant variety, Delwiche Commando.

are summarized in table 2. Since the susceptible plants were discarded in the F_2, segregation would be expected to be in a ratio of 1 resistant progeny to 2 segregating. According to the Chi-square test, 15 of the 16 crosses showed a good fit to the 1:2 ratio. There were a few progenies that were completely susceptible, indicating that an occasional susceptible plant escaped in 1943, as has already been pointed out with respect to the susceptible controls. There was a total of 10 such progenies, 3 in one cross, 2 in 3 other crosses, and 1 in a sixth cross. In each of these Merit or Penin was the susceptible parent.

The reaction of F_4 families from heterozygous F_2 plants in the various crosses may be seen in table 3. Excluding cross C127, 13 of the 15 crosses showed a good fit for a 1:2:1 ratio, and 53 of the 59 groups of families from individual F_2 plants were a good fit to a 1:2:1 ratio. In the C127 cross which had Penin as the susceptible parent there was a large number of very late-maturing segregates. Since near-wilt symptoms do not appear commonly until very near the physiological maturity of the plant (*9*), it was impossible to make accurate readings in these late-maturing segregates because low average temperature retarded development of near-wilt. Furthermore, secondary resistance derived from Penin may have caused some masking of susceptibility. In the other two crosses which deviated significantly from a 1:2:1 ratio, the deficiency was in the susceptible class. It is possible that classification in these was affected by retarded near-wilt development, although such was not so evident at the time as in the case of C127.

TABLE 2.—*Reactions of F_3 progenies on near-wilt soil near Waupun, Wis., in 1944; susceptible plants discarded in F_2*

Susceptible parent	Cross number or variety	Total progenies	Progenies in class indicated			Chi-square for goodness-of-fit to 1:2 ratio
			Resistant	Segregating	Susceptible	
		Number	*Number*	*Number*	*Number*	
Perfected Wales	C125	22	6	16		0.14
	C129	25	9	16		.01
	C132	21	6	15		.05
	C135	10	3	7		[1]
	C138	5	1	4		.03
	Perfected Wales	[2] 70	2		68	
	Wisconsin Perfection	33	1		32	
Merit	C126	23	3	18	[3] 2	2.63
	C130	13	4	7	[3] 2	[1]
	C133	20	6	14		.01
	C136	15	2	10	[3] 3	.84
	C139	16	7	8	[3] 1	.68
	Merit	68	6		62	
	Wisconsin Perfection	28	0		28	
Penin	C127	29	8	19	[3] 2	.04
	Wisconsin Perfection	60	1		68	
Wisconsin Perfection	C128	37	6	31		4.14*
	C131	33	9	24		.31
	C134	5	0	5		1.23
	C137	4	0	4		.78
	C140	5	1	4		.03
	Wisconsin Perfection	146	5		141	

*Significant, 5 percent.
[1] Value for Chi-square less than 0.01.
[2] Individual plants tabulated in susceptible checks.
[3] Probably from susceptible escapes in the F_2 progeny in 1943.

TABLE 3.—*Reactions of F_4 progenies from heterozygous F_2 plants on near-wilt soil near Waupun, Wis., in 1944*

Susceptible parent	Cross number or variety	F_2 plants	Total progenies	Progenies in class indicated			Chi-square for goodness-of-fit to 1:2:1 ratio
				Resistant	Segregating	Susceptible	
		Number	*Number*	*Number*	*Number*	*Number*	
Perfected Wales	C125	3	57	16	22	19	3.28
	C129	3	68	20	32	16	.71
	C132	5	125	38	69	18	7.75*
	C135	2	44	17	19	8	4.50
	C138	4	72	31	26	15	12.67**
	Perfected Wales		[1] 283	8		275	
	Wisconsin Perfection		281	5		276	
Merit	C126	2	44	11	23	10	.14
	C130	4	90	28	41	21	1.80
	C133	6	117	23	60	34	2.15
	C136	4	75	22	40	13	2.49
	C139	3	58	15	30	13	.21
	Merit		283	34		249	
	Wisconsin Perfection		403	5		398	
Penin	C127	11	228	94	94	40	32.60**
	Penin		241	9		232	
	Wisconsin Perfection		174	1		173	
Wisconsin Perfection	C128	4	126	28	63	35	.78
	C131	6	90	24	44	22	.13
	C134	5	123	30	64	29	.22
	C137	4	92	28	45	19	1.80
	C140	4	93	29	46	18	2.61
	Wisconsin Perfection		627	37		590	

*Significant, 5-percent; ** significant, 1-percent.
[1] Individual plants tabulated in susceptible checks.

The only F_1 which was tested on diseased soil was that of cross C141, tested in 1944. On July 24, the last date on which readings were made that season, all 26 plants of C141 were healthy while 60 plants of the resistant parent (738–49/I) on one side of the F_1 were healthy, and 50 plants of the suceptible parent (lot 23) on the other side had wilted and 4 were healthy. In this case, then, resistance was completely dominant in the F_1, as would be expected from the type of segregation in F_2.

It has been pointed out that, environment being favorable, the onset of the near-wilt disease in the field is delayed until shortly before the plant approaches physiological maturity. Since canning peas usually mature quite rapidly and since the plants dry out as the seeds become ripe, the normal period for the development of the disease is short. Moreover, environmental conditions have a marked effect on disease development. In 1945 the season was moist and cool throughout and very little near-wilt developed on the same plot where excellent results were obtained in 1943 and in 1944 with the exception of the late-maturing progenies.

Resistant selections from cross C140 (table 3) were crossed with several susceptible varieties and the F_1 was grown on near-wilt-free soil in the field in 1945. The F_2 progenies were planted on the Waupun near-wilt plot in 1946. Near-wilt developed abundantly, but severe drought and hot weather preceded appearance of symptoms and so hastened plant maturity that it was impossible to distinguish signs of near-wilt from those of premature ripening. An attempt was made to record the healthy and diseased plants in the F_2 progenies, although accurate classification was not possible. The results are presented in table 4. Twelve of 19 progenies did not show a good fit to a 3:1 ratio.

It became apparent, after field trials for four successive seasons on the Waupun plot that seasonal environment so influenced the expression of near-wilt that accuracy of disease readings might be impaired. In two seasons, 1943 and 1944, conditions were favorable for the full expression of near-wilt. In the following seasons readings were impaired in one case by premature maturation caused by excessive drought and heat, and in the other case by too low average temperature. In 1947, an attempt was made to intensify disease development by planting seed in heavily inoculated field plots at Madison, Wis. Concentrated inoculum of the near-wilt organism was applied in four ways: (1) Heavily infested soil in the row under the seed; (2) heavy liquid suspension of spores and mycelium in the row under the seed; (3) application of a suspension after (2) to the soil near the roots when plants were 6 inches high, and (4) when plants were 12 inches high. No diseased plants resulted even in susceptible progenies. Very few diseased plants developed in a susceptible variety on the Waupun plot the same year. The uncertainty of field analysis of progenies segregating for resistance thus became a decidedly limiting factor in this study and in a concomitant breeding program of developing near-wilt-resistant varieties. For this reason the possibilities of greenhouse testing were explored.

TABLE 4.—*Reaction of the F_2 progenies on near-wilt soil near Waupun, Wis., in 1946 and in greenhouse tests; and of F_3 progenies in greenhouse tests*

Cross	Parents	F_2 in field, Waupun, 1946			F_2 in greenhouse			F_3 in greenhouse		
		Resistant plants	Susceptible plants	Chi-square for goodness-of-fit to 3 : 1 ratio	Resistant plants	Susceptible plants	Chi-square for goodness-of-fit to 3 : 1 ratio	Resistant plants	Susceptible plants	Chi-square for goodness-of-fit to 5 : 3 ratio
		Number	*Number*		*Number*	*Number*		*Number*	*Number*	
C150	Alsweet × C140	143	85	17.69**				20	18	1.19
C151	737–1 × C140	259	148	27.43**	16	4	0.07	72	52	.86
C152	534–12 × C140	459	300	84.64**	16	4	.07	43	22	.23
C156	741–33 × C140	387	75	18.47**				123	12	45.94**
C157	532–6 × C140	145	50	.02	14	4	[1]	50	27	.10
C158	733–7 × C140	211	61	.83	16	4	.07	50	31	[1]
C159	739–22 × C140	243	54	7.00**				22	24	3.62
C160	Lot 10 × C140	339	190	33.04**	21	2	2.45	64	45	.51
C161	Lot 11 × C140	175	25	16.01**				43	19	.97
C162	Lot 12 × C140	56	19	[1]						
C163	Lot 13 × C140	109	30	.69				30	16	.05
C164	Canner King × C140	217	67	.23				39	13	2.95
C165	Pride × C140	91	39	1.48				34	18	.08
C166	Lot 23 × C140	126	60	4.85*	22	3	1.61	47	38	1.59
C167	733–23 × C140	75	4	15.70**				40	17	1.12
C168	733–42 × C140	41	1	10.29**				9	9	.73
C169	Alderman × C140	683	115	47.16**	96	32	[2]	16	9	[1]
C171	Prince of Wales × C140	251	76	.45				47	16	3.44
C174	744–12 × C140	339	139	4.03*						
C178[3]	Loyalty × Delwiche Commando				52	17	[1]			

*Significant 5-percent; **significant 1-percent.
[1] Value for Chi-square less than 0.01.
[2] Total of 2 trials (50 : 10 and 46 : 22) made 1 month apart.
[3] Cross made in 1946.

GREENHOUSE TESTS

It was shown by Virgin and Walker (*9*) that near-wilt develops more slowly than wilt in the greenhouse over a range of soil temperatures. Schroeder and Walker (*4*) showed that as the temperatures of wilt-inoculated quartz sand increased, the disease increased up to about 28° C. and as it approached the optimum, varieties perfectly resistant to wilt in the field succumbed. In setting up greenhouse tests for near-wilt it became necessary to determine a set of controlled and readily reproducible conditions under which repeated tests could be made. The numerous tests made with Delwiche Commando and Wisconsin Perfection varieties to determine a standard technique will not be presented in detail. Soil as a substrate was found to be unreliable, and rapid disease development was not attained. Quartz sand inoculated by the method of Schroeder and Walker (*4*) did not give rapid or consistent results with near-wilt. Increase of temperature toward the optimum (28°) resulted in erratic disease development in Delwiche Commando. More consistent results were secured with plants grown in sand until 2 inches high, removed for inoculation, and replanted. Dipping the roots in concentrated inoculum did not give as rapid nor as consistent results as clipping the taproot uniformly to 1-inch length while the entire root system was immersed in the inoculum. This method gave the most satisfactory results when the sand temperature was kept at 21°.

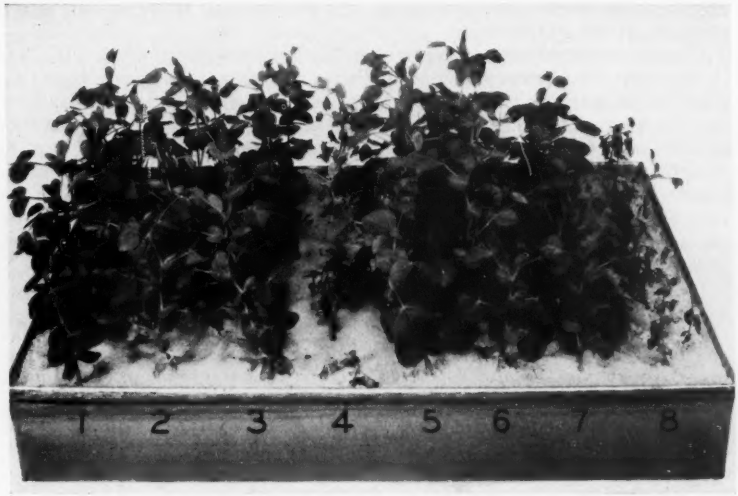

FIGURE 2.—Peas grown in sterilized quartz sand at 21° C. and inoculated with *Fusarium oxysporum* f. *pisi* race 2. Odd-numbered rows, Delwiche Commando, even-numbered, Wisconsin Perfection. Rows 1 and 2, inoculated by dipping roots in spore suspension; rows 3, 4, 7 and 8, inoculated by clipping taproots to 1 inch with entire root system immersed in suspension; rows 5 and 6, uninoculated.

A comparison of the last two methods is illustrated in figure 2. The cut-root dip method was used for all subsequent greenhouse progeny tests.

Since the 1946 field trials with F_2 populations did not confirm those of 1943, remnants of F_2 seed were planted in the field plot in 1947 and seed of single F_2 plants was saved. Inasmuch as near-wilt did not appear in the 1947 plot, no elimination of susceptible plants occurred. Since limitation of space prohibited testing each individual 1947 plant progeny in the greenhouse, two seeds were taken from each F_2 plant and bulked to form an F_3 population representing each F_2 plant. If the two seeds selected in the heterozygous families were at random, then segregation based on a single-gene difference between parents would be expected in a ratio of 5 resistant to 3 susceptible individuals. Some F_2 seeds from a few of the crosses were still available, as were larger amounts from crosses C169 and C178. These lots were also tested by the greenhouse method.

The results of the greenhouse trials are presented in table 4. Seventeen families were tested in the F_2 at Waupun in 1946 and in the F_3 in the greenhouse. In the 1946 season, which was unfavorable for disease evaluation 11 families deviated significantly from the expected 3:1 segregation. Under the much more exacting greenhouse test, 16 of the families segregated close to the expected 5:3 ratio. The deficiency in susceptible segregates in 741–33 × C140 in both field and greenhouse has not been explained. In seven families tested in the F_2 in the field in 1946 and in the greenhouse, five deviated

significantly from the expected 3 to 1 in the field while all showed a good fit in the greenhouse.

The greenhouse results confirmed the field results of 1943 and 1944 in showing that crosses of susceptible varieties with a resistant parent which was extracted from one of the original crosses yielded confirming 3:1 segregation in the F_2 and 5:3 in the F_3. They also showed conclusively that a relatively simple greenhouse technique can be used to differentiate individuals in segre gating progenies into discontinuous near-wilt-resistant and near-wilt-susceptible classes. Field tests for this purpose are often unreliable because of the short period of complete near-wilt expression and the great liability that unfavorable environment will preclude accurate differentiation.

DISCUSSION

There are several factors which complicate the separation of pea plants into resistant and susceptible classes on the basis of reaction to near-wilt. One of these is the presence of a secondary type of resistance in some varieties such as Rogers K and Horal. This type of resistance will often carry through when conditions for disease development are below optimum, and under favorable conditions will retard development of the disease as compared to that in varieties like Wisconsin Perfection which do not possess the secondary resistance.

With favorable conditions for disease development a small percentage of diseased plants appeared consistently in the resistant parent line. The symptoms, however, differed from those of near-wilt in that there was more cortical rotting of the roots and slower dying of the leaves in contrast to the rapid unilateral yellowing, wilting, and dying of typically diseased plants. On the other hand, in all field trials thus far there have always been occasional escapes in susceptible varieties under the most favorable conditions for near-wilt. Diseased plants in resistant progenies and escapes in susceptible varieties have been recognized in pure-line material. Undoubtedly, in combination with other factors they have affected the accuracy of classification of segregating progenies.

The most disturbing influence on accuracy of classification has been that of environmental factors. The most important of these factors has been cool weather which favored mild development of the disease and a large number of escapes. While the optimum for wilt in the field is 22° C., that for near-wilt is from 24° to 28° (*9*). Near-wilt develops more slowly than wilt when each are at their respective optimum. The delayed appearance of near-wilt is also influenced by the fact that varieties differ in the rate of disease development, the disease progressing more slowly in later-maturing varieties. The high temperature needed for optimum disease development and the association of the latter with approaching host maturity shortens the period during which symptoms are expressed. This is illustrated in figure 1, where it is to be noted that although all plants of the susceptible variety were dead, they had produced as many and as large pods as the resistant variety before they succumbed. The first symptoms in this case were noted during the first week in July and when the photograph was made on July 12 all susceptible plants were dead. A hot, dry season like that of 1946 may disturb classification of

plants as badly as a cool season because healthy plants mature and die so quickly after the appearance of near-wilt that it becomes impossible to distinguish those killed by drought from those killed by near-wilt.

The method of testing used in the greenhouse also has disadvantages. Besides the obvious limitation in amount of material that can be handled, resistance may break down somewhat with the treatment required to produce satisfactory elimination of susceptible plants. The difference between the resistant and susceptible classes was too small at 24° C. while at 21° it was usually sufficient for practical purposes (fig. 2). However, even at the latter temperature some variation in severity of disease occurred between runs, but the reasons are not understood. To compensate for this variation, classification of segregating material was always made in reference to known resistant and susceptible varieties grown in the same pan.

General observations and surveys of the canning crop in Wisconsin have shown variations in amount of near-wilt from season to season in those areas where it occurs in conformity with the variations recorded here. Although several recent seasons have been unfavorable for its development, near-wilt remains a major disease of the canning crop in Wisconsin and seasonal variation in weather may provide more nearly optimum conditions in future years. Since the resistance now available in line 738–49, unlike wilt resistance, does not occur naturally in any of the commercial varieties tested, desirable types with near-wilt resistance will have to be developed by hybridization. Since the high degree of resistance in the 738–49 line is shown to be controlled by a single dominant gene, greatest progress can probably be made with the application of the controlled greenhouse testing technique described herein for the screening of breeding materials for near-wilt resistance.

SUMMARY

A high type of resistance to near-wilt of garden pea, incited by *Fusarium oxysporum* f. *pisi* race 2, is described. This resistant character was apparently inherited from one of the Admiral selections used as parents of strain 732–66–1 in which resistance was discovered.

Evidence from the F_2, F_3, and F_4 generations of one set of crosses, and from the F_2 and F_3 generations of a second set of crosses involving resistant selections from the first group shows that the inheritance of resistance is dependent upon a single dominant gene.

Difficulties in testing for resistance to the disease under field conditions over a period of years are indicated. A method of testing with controlled environment and inoculation in the greenhouse is described.

LITERATURE CITED

(1) Anonymous
1945. wilt-resistant canning pea named for prof. e. j. delwiche. Canner 101 (15): 11.

(2) Jones, F. R., and Linford, M. B.
1925. pea disease survey in wisconsin. Wis. Agr. Expt. Sta. Res. Bul. 64, 31 pp., illus.

(3) Linford, M. B.
1928. a fusarium wilt of peas in wisconsin. Wis. Agr. Expt. Sta. Res. Bul. 85, 44 pp., illus.

(4) SCHROEDER, W. T., and WALKER, J. C.
1942. INFLUENCE OF CONTROLLED ENVIRONMENT AND NUTRITION ON THE RESISTANCE OF GARDEN PEA TO FUSARIUM WILT. Jour. Agr. Res. 65: 221-248, illus.
(5) SNYDER, W. C.
1933. A NEW VASCULAR FUSARIUM DISEASE OF PEAS. Science 77: 327.
(6) ——— and HANSEN, H. N.
1940. THE SPECIES CONCEPT IN FUSARIUM. Amer. Jour. Bot. 27: 64–67.
(7) ——— and WALKER, J. C.
1935. FUSARIUM NEAR-WILT OF PEA. Zentbl. f. Bakt. [etc.] (II) 91: 355–378, illus.
(8) VIRGIN, W. J., and WALKER, J. C.
1938. NO VARIETY OF CANNING PEA IS IMMUNE TO NEAR-WILT. *In* Wis. Agr. Expt. Sta. Bul. 440 (Part II), pp. 50–52, illus.
(9) ——— and WALKER, J. C.
1939. RELATION OF TEMPERATURE AND MOISTURE TO NEAR-WILT OF PEA. Jour. Agr. Res. 59: 591–600, illus.
(10) WADE, B. L.
1929. INHERITANCE OF FUSARIUM WILT RESISTANCE IN CANNING PEAS. Wis. Agr. Expt. Sta. Res. Bul. 97, 32 pp., illus.
(11) WALKER, J. C.
1931. RESISTANCE TO FUSARIUM WILT IN GARDEN, CANNING AND FIELD PEAS. Wis. Agr. Expt. Sta. Res. Bul. 107, 15 pp., illus.
(12) ———, DELWICHE, E. J., and HARE, W. W.
1944. A MAJOR GENE FOR RESISTANCE TO NEAR-WILT IN PEA. (Abstract) Phytopathology 34: 1013.
(13) ———, and SNYDER, W. C.
1942. PEA WILTS AND ROOT ROTS. Wis. Agr. Expt. Sta. Bul. 424, 16 pp., illus. (Revised.)

SOME ASPECTS OF THE LONGITUDINAL GROWTH OF BROMEGRASS FRUITS [1]

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INTRODUCTION

Although the growth of many plants and plant organs has been extensively studied, little research seems to have been directed toward the growth of grass fruits. This aspect of growth is important in view of the large economic value of the fruits of the Gramineae and the possible use of such information in agricultural practice.

In 1920 Harlan (*4*),[3] working on Hannchen barley, made a study of the growth of grass fruit from flowering to maturity. He found that most of the growth of the kernel was made in the first 3 days after pollination. The growth curve showed a sharp rise at first, a leveling, and then a small dip at the end. Half the growth in length was made from the second to the fourth day after fertilization, and in 7 days maximum length had been attained. After the peak was reached, there was a gradual falling off toward maturity.

Pope (*8*) reported that in barley kernels growth limitation is largely due to starch congestion. Such large amounts of starch are stored in the endosperm cells that mitotic division ceases. Deposition of starch in amounts sufficiently large to hinder cell division places a limit on the maximum size of the kernel.

Harlan and Pope (*5*), in a study of growth in immature barley kernels removed from the plant, noted that the endosperm and embryo grew at least 8 days after the heads had been harvested. The explanation offered for this was that food materials passed from the severed culm into the developing fruit. The fruits were almost 4 mm. long 2 days after pollination, 6 mm. long after 3 days, 8 mm. after 4 days, 8.8 mm. after 5 days, and by the end of the seventh day the plumule and radicle were visible.

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[2] The writer wishes to thank Victor R. Gardner, director of the Michigan Agricultural Experiment Station, and Dean Howard C. Rather, of the Basic College, for encouragement during the pursuit of this project. The writer is indebted also to Prof. Roy E. Decker, head of the Farm Crops Department, Prof. Eugene H. Lucas, Prof. Carter Harrison, and Prof. Irma M. Felber for needed facilities; to Prof. Boyd R. Churchill for the use of plant materials from his experimental plots; and to Prof. Hubert M. Brown for helpful criticisms of the manuscript. The Botany Department under Dr. E. A. Bessey furnished some needed materials.

[3] Italic numbers in parentheses refer to Literature Cited, p. 256.

Booth (*1*) reported that growth in length of the oat kernel was most rapid during the first 6 days after pollination. It then became more gradual until the fifteenth day, when the maximum was reached.

MATERIALS AND METHODS

Fruits were collected during 1945 and 1946 from 50 mature plants of smooth bromegrass (*Bromus inermis* Leyss.) of the Achenbach strain growing in the Farm Crops nursery at East Lansing. The plants used represented a fairly random sample of the hundreds of plants in the nursery. Individual spikelets were tagged when the basal floret on each came into anthesis. Tagging proceeded during most of the flowering period of the plots, and specimens were collected each day after the first day of tagging, thus "a 1-day-old fruit" was collected 24 hours after pollination. It is highly probable, as pointed out by Nielsen (*7*), that fertilization takes place 15 to 18 hours after pollination. Although the author (*6*) had previously shown that, under favorable conditions, the anthesis of florets in a bromegrass spikelet proceeds evenly, the basal floret opening first, followed the next day by the one above it, and so on apically until the sterile floret is reached, the variable climatic conditions of 1945 and 1946 made it unsafe to proceed on this basis. Accordingly, only the basal floret or fruit was removed in each case. As these were collected, the fruits were immersed in strong Navashin (Craf) solutions, as outlined by Sass (*9*). Evacuation of the air in the fruits was followed by direct measurements with a millimeter rule placed under the 32-mm. objective of a dissecting microscope. Fruits were measured from their base to their apex, omitting the protruding hairs.

PRESENTATION OF RESULTS

In 1945 measurements were made on a tentative basis and were carried only up to the fifteenth day after pollination. The measurements for 191 fruits for this period are shown in table 1. No 9-day-old fruits were obtained in this year. The length of the 1-day-old fruits was approximately that of the ripe ovary, which is understandable when one considers that fertilization occurred 6 to 9 hours before gathering.

During the first 15 days the fruit grew from a length of 1.5 mm. to 9.5 mm. Growth was slow at first, only 0.4 mm, or 5 percent, of the total growth occurring in the first 4 days (table 2). From the fourth

TABLE 1.—*Average measurements of the length of developing bromegrass fruits in 1945*

Day No.	Fruits	Average length	Day No.	Fruits	Average length
	Number	*Mm.*		*Number*	*Mm.*
1	16	1.5	9	0	
2	15	1.6	10	15	8.9
3	15	1.7	11	18	9.0
4	14	1.9	12	15	8.7
5	12	5.4	13	18	8.8
6	14	6.5	14	6	9.5
7	16	7.6	15	5	9.5
8	12	7.8			

TABLE 2.—*Periodic increases in length, by percentages, of bromegrass fruits in 1945*

Days	Increase in growth	Percentage of total increase	Days	Increase in growth	Percentage of total increase
	Mm.			*Mm.*	
1–4	0.4	5.0	10–15	.6	7.5
4–5	3.5	43.75			
5–10	3.5	43.75	Total	8.0	

to the fifth day, however, growth was very rapid, that is, 3.5 mm., or 43.75 percent, of the total gain in length. Similar growth was made from the fifth to the tenth day, but only 0.6 mm., or 7.5 percent, was made the last 5 days.

The upper curve of figure 1 shows the growth of the 191 fruits. This curve, based on the data in table 1, shows that early slow growth was followed by a period of greatly accelerated increment and then by a gradual slowing down.

In 1946, a more intensive study was conducted on 225 fruits. The measurements of these fruits are given in table 3. These were carried through to the twentieth day but some days are not represented. In most cases failure to collect was due to the fact that the desired fruits did not develop. Some inconsistencies in average length on certain days may be noted. These have been due to sampling errors or to fluctuations in climatic conditions, or both. The fruits grew from 1.6 mm. to 9.8 mm. and at maturity measured only 8.5 mm. The decrease in length at maturity may be explained as a dehydration phenomenon normally accompanying the final maturing of the fruit.

Table 4 reveals that, as in 1945, growth was slow at first. During the first 3 days the fruits grew 0.7 mm., or 8.5 percent, of their total length. From the third to the fifth day, they grew 6.5 mm., or 79.3 percent; from the fifth to the ninth day they grew only 1.0 mm., or 12.2 percent, while from the ninth to the twentieth day they showed a negative growth of −1.0 mm.

The lower curve of figure 1 demonstrates the progress in growth in 1946 fruits and shows the same general features exhibited by the 1945 curve. It is apparent that acceleration in the rate began earlier in 1946 than in 1945 and proceeded somewhat more rapidly during the early period. In comparing the two curves with the percentages in tables 2 and 4, it can be seen that in 1945, 48.75 percent of the growth

TABLE 3.—*Average measurements of the length of developing bromegrass fruits in 1946*

Day No.	Fruits	Average length	Day No.	Fruits	Average length
	Number	*Mm.*		*Number*	*Mm.*
1	10	1.6	11	8	8.4
2	1	1.7	12	4	8.7
3	5	2.3	13	0	
4	40	5.1	14	68	9.3
5	14	8.8	15	0	
6	15	9.6	16	17	9.5
7	11	9.6	17	0	
8	0		18	0	
9	13	9.8	19	0	
10	13	7.8	20	6	8.5

had occurred by the end of the fifth day. In 1946, by the end of the fifth day 87.8 percent of the growth had occurred.

Since weather conditions affect the rate of growth (*2*), a summary of the weather conditions prevailing in the East Lansing area in June and July of 1945 and 1946 is given (table 5). The data show that

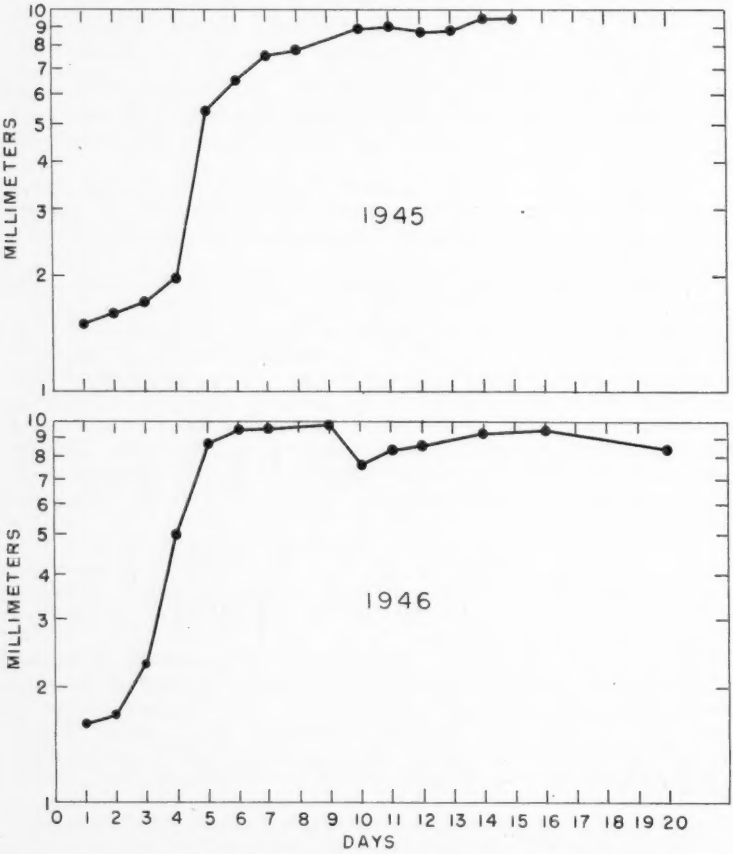

FIGURE 1.—Growth curves of bromegrass fruits in 1945 and 1946.

during these 2 months of fruit development, the temperature was higher in 1946 than in 1945, if one adds the accumulated excess for the 2 months in each case. There was also less deficiency in precipitation and a higher percentage of the possible sunshine in 1946 than in 1945. These differences in climatic conditions may account for the differences in the growth curves.

TABLE 4.—*Periodic increase in length, by percentages, of bromegrass fruits in 1946*

Days	Increase in growth	Percentage of total positive increase	Days	Increase in growth	Percentage of total positive increase
	mm.			*mm.*	
1-3	0.7	8.5	9-20	−1.0	−12.2
3-5	6.5	79.3			
5-9	1.0	12.2	Total	8.2	

TABLE 5.—*Summary of weather conditions in June and July of 1945 and 1946 at East Lansing, Mich.*

Item	1945		1946	
	June	July	June	July
Accumulated excess (+) or deficiency (−) in mean temperature since first day of month ..... °F.	+21	+8	+36	+2
Excess (+) or deficiency (−) in precipitation for the month as compared with normal rate ..... inches	+.19	−1.02	−1.03	−2.85
Accumulated excess (+) or deficiency (−) since Jan. 1 ..... inches	−.27	−.15	−.08	−.09
Mean percentage of relative humidity:				
7:30 a. m.	80	80	76	74
1:30 p. m.	56	50	55	40
7:30 p. m.	62	55	61	46
Total hours actual sunshine	265	336	268	386
Percent of possible hours sunshine	58	72	58	83

DISCUSSION OF RESULTS

The growth curves of bromegrass fruits in 1945 and 1946 follow closely the pattern shown for other fruits by Gustafson (*3*), for barley by Harlan (*4*), and for oats by Booth (*1*)—Both curves show the initial slow period, the tremendous increase in rate thereafter, followed by a slackening. In barley most of the fruit growth was made in the first 3 days, in oats the greatest length was attained by the sixth day, while in bromegrass most of the growth was made by the end of the fifth day. Different climatic conditions might, of course, produce a different result.

Some of the reasons usually given for the four distinct periods in the growth curve, slow growth, rapid growth, leveling-off growth, and negative growth, are: (1) an increase or decrease in cell multiplication; (2) interference with mitosis by starch accumulation in the endospermal cells; (3) the absorption or loss of water in the tissues of the fruit; and (4) genetic factors. An anatomical investigation now under way on the embryological development of the species will attempt to correlate the tissue development with the growth curve and may give some explanation for the periods.

SUMMARY

Bromegrass fruits were measured in 1945 and 1946 and growth curves were established for those 2 years under the prevailing climatic conditions. The growth curves agree closely with those of other fruits. In bromegrass most of the longitudinal growth in the fruit had occurred by the end of the fifth day.

LITERATURE CITED

(1) Booth, E. G.
1929. daily growth of the oat kernel and effect on germination of immaturity and controlled low temperatures. Minn. Agr. Expt. Sta. Tech. Bul. 62, 42 pp., illus.
(2) Gregory, F. G.
1926. the effect of climatic conditions on the growth of barley. Ann. Bot. [London] 40: [1]–26, illus.
(3) Gustafson, F. G.
1926. growth studies on fruits. Plant Physiol. 1: 265–272, illus.
(4) Harlan, H. V.
1920 daily development of kernels of hannchen barley from flowering to maturity at aberdeen, idaho. Jour. Agr. Res. 19: 393–430, illus.
(5) ——— and Pope, M. N.
1926. development in immature barley kernels removed from the plant. Jour. Agr. Res. 32: 669–678, illus.
(6) Knobloch, I. W.
1944. development and structure of bromus inermis leyss. Iowa State Col. Jour. Sci. 19: 67–98, illus.
(7) Nielsen, E. L.
1947. macrosporogenesis and fertilization in bromus inermis. Amer. Jour. Bot. 34: 431–433, illus.
(8) Pope, M. N.
1932. the growth curve in barley. Jour. Agr. Res. 44: 323–341, illus.
(9) Sass, J. E.
1940. elements of botanical microtechnique. 222 pp., illus. New York.

THE EFFECT OF SUNLIGHT ON THE ASCORBIC ACID CONTENT OF STRAWBERRIES[1]

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INTRODUCTION

Although it is generally realized that environmental factors may influence the nutritive value of fruits and vegetables, there has not been a great deal of research reported to show the extent to which climatic variants may exert their effect. The work reported in this paper is concerned with the relationship between sunlight and the ascorbic acid content of strawberries.

Reports in the literature on the ascorbic acid-sunlight relationship for strawberries are rather conflicting, although they are agreed that there is such a relationship. McCrory (*3*)[3] found that long-stemmed fruits were higher in ascorbic acid than those with shorter stems, and that fruit exposed to sun was higher than that shaded by leaves. He also reported that fruit harvested on clear days had a higher ascorbic acid content than those harvested on cloudy days. However, these observations were not supported with data. Hansen and Waldo (*2*) studied the problem by shading entire plants, or the berries alone, with muslin bags and concluded that under the former condition a much lower amount of ascorbic acid was synthesized. The effect of shading the berries alone was not so great. However, the statistical significance of these observations could not be determined by the data presented. Ezell and associates (*1*) found that when entire plants or only the berries were shaded, less ascorbic acid was accumulated than with full illumination. These workers used one and four thicknesses of cheesecloth for the plot-shading experiments and brown manila bags for the shading of the fruit alone. They also related climatological data published by the United States Weather Bureau to the seasonal fluctuations of the strawberry ascorbic acid and deduced that the amount of sunlight for approximately 1 week prior to harvest is operative on the ascorbic acid production.

In a survey of the ascorbic acid content of 81 varieties of strawberries in 1946 Robinson *et al.* (*4*) found a statistically significant difference of 15 percent in the average ascorbic acid content (fresh-weight basis)

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[2] The author gratefully acknowledges the loan of the light recorders of the United States Plant, Soil, and Nutrition Laboratory at Ithaca, N. Y., and the valuable cooperation of Dr. G. F. Somers of that laboratory.

[3] Italic numbers in parentheses refer to Literature Cited, p. 262.

of the fruit grown at two different locations. Strawberries grown on Dunkirk fine sandy loam 5 miles north of Geneva contained more ascorbic acid than those grown on Dunkirk silty clay loam at Geneva.

EXPERIMENTAL PROCEDURE

Two types of experiments were undertaken; one involved a comparison of the ascorbic acid content at the two locations mentioned above, and the other involved a study of the influence of shading upon the ascorbic acid content of strawberries. One light recorder was installed at each of the two locations, i. e., at Geneva and at the farm 5 miles north of Geneva, in order to obtain a comparison of the amount of illumination at the two localities. Nine varieties of strawberries grown at each location were compared twice during the season.

The integrating light recorders used are a modification of the instrument described by Sprague and Williams (*6*). The same principles of operation are involved, but the details of the electrical circuit and recorder are somewhat different. The phototube housing is composed of a brass case on top of which is mounted an opal glass globe. The top of the brass case has an aperture containing 1-inch-square glass filters and a ground-glass plate. The filters are Corning Nos. 3962 and 3850, which in combination transmit a very broad band of light, principally in the visible portion of the spectrum with a maximum at about 5,000 Å. Some ultraviolet light longer than a wave length of 3,700 Å. is transmitted and there is a small amount of transmission beyond 7,500 Å. (up to 10,000 Å). This combination, in conjunction with RCA 922 phototube, gives two peaks in the maximum sensitivity curve. One maximum is at about 4,000 Å. and the other is at about 6,000 Å.

The shading experiments were all made at Geneva. The effect of shading only the fruit was studied by placing white parchment paper bags over them a few days after the blossoming stage. The paper was found to reduce the illumination to 52 percent of that of full sunlight, as measured by the integrating light recorder. One plot of berries was entirely covered by a cheesecloth cubical (12′ x 12′ x 12′) that enclosed a light recorder as well. The amount of light reaching the strawberries thus shaded was 43 percent of that reaching the fully exposed plants. The variety Premier (Howard 17) was used for all of the shading tests.

In all cases, the fruit was picked between 8 and 9 o'clock in the morning and analyses were completed the same day. Ascorbic acid analyses were made on individual berries by the xylene-extraction modification of the indophenol method (*5*). Although ascorbic acid analyses were made on individual fruits each day, only the averages for each day with the standard errors are reported here. Statistical analyses were made by the paired comparison method of Student (*7, 8*). On the same day, the averages of each group in the shading test, and each variety in the soil test, were used for the pairing.

EXPERIMENTAL RESULTS

The fruits ripened in the cheesecloth cubical were invariably lower in ascorbic acid content than those ripened with either the leaves or the whole plants exposed to full sunlight (table 1). Each of these

comparisons was highly significant (odds greater than 4,999 and 9,999 to 1, respectively). The comparison between the fruit ripened in complete sunlight and that ripened with only the fruit shaded showed no significant difference. The data are presented on the fresh weight basis so that they can be more readily compared with other data in the literature. Calculation of the data for the shading experiments on either the fresh or the dry weight basis made no difference in the conclusions drawn from these tests.

The relation between the day to day variations in ascorbic acid and the amount of sunlight is shown in figure 1, where the ascorbic acid content of fruits from fully exposed plants (table 1) are compared with the daily sunlight records. The sunlight data are presented in graphical form, with the sunniest day, June 20, represented as 100 percent. The ascorbic acid level in the fruit corresponded fairly well with the relative intensity of the sunlight on the fifth or sixth day prior to ripening. The date of harvest is plotted 5 days ahead of the sunlight record in the figure to emphasize this relationship.

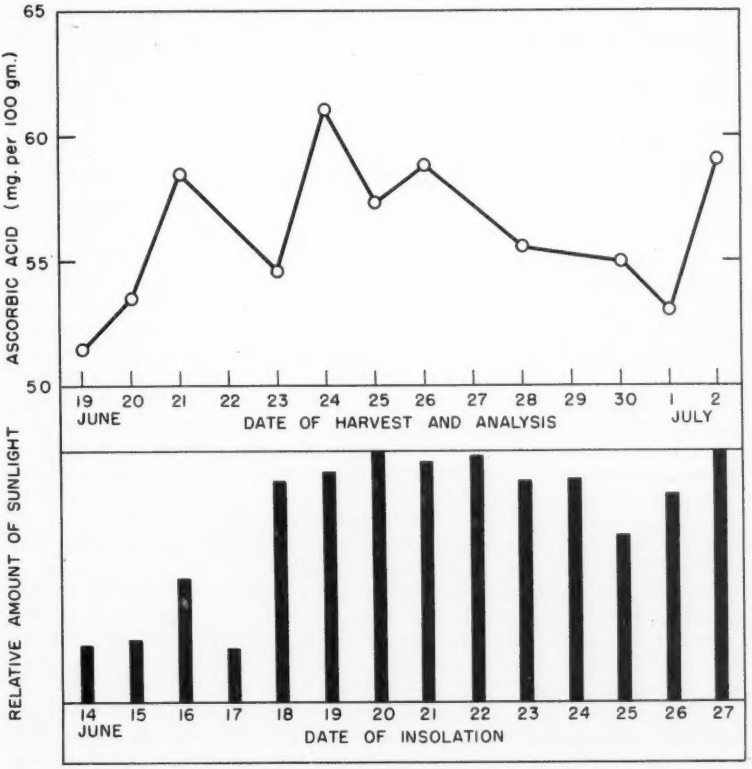
ASCORBIC ACID (mg. per 100 gm.)
65
60
55
50
19 20 21 22 23 24 25 26 27 28 29 30 1 2
JUNE
DATE OF HARVEST AND ANALYSIS
JULY
RELATIVE AMOUNT OF SUNLIGHT
14 15 16 17 18 19 20 21 22 23 24 25 26 27
JUNE
DATE OF INSOLATION

FIGURE 1.—Relation between amount of sunlight and ascorbic acid content of strawberries grown at Geneva, N. Y., 1947.

TABLE 1.—*Effect of shading on the ascorbic acid content of strawberries* [1]

Date, 1947	Full exposure to sun light		Fruit shaded, leaves exposed		Leaves and fruit shaded	
	Berries	Ascorbic acid	Berries	Ascorbic acid	Berries	Ascorbic acid
	Number	*Mg. per 100 gm.*	*Number*	*Mg. per 100 gm.*	*Number*	*Mg. per 100 mg.*
June 20	40	53.59±1.21	7	46.90±1.39		
June 21	10	58.55±1.74	10	47.81±1.50	3	40.32±1.95
June 23	25	54.64±1.79	25	56.95±2.19	8	42.46±3.19
June 24	10	61.06±3.56	10	58.12±1.45		
June 25	10	57.41±3.26	10	65.56±3.66	10	34.35±1.64
June 26	12	58.66±3.28	12	60.81±3.63	12	40.95±2.47
June 28	8	55.78±3.41	8	49.04±1.63	8	40.27±1.66
June 30	11	55.22±2.06	11	53.70±2.74	12	32.28±1.18
July 1	12	53.08±2.44	12	53.40±3.19	12	29.82±1.83
July 2	15	59.27±2.09	15	50.46±1.98	15	30.42±2.06
Average		56.72		54.28		36.36

[1] Data expressed on fresh weight basis, variety, Premier (Howard 17).

The comparison between the two locations mentioned above, in which nine varieties of strawberries were used, again gave significant differences in ascorbic acid content on the fresh weight basis (table 2). However, the difference in amount of sunlight was insignificant. There was 4.3 percent less sunlight at the location where the higher ascorbic acid content was found. Examination of the dry weight data showed that the berries grown on the sandy soil had a higher total solids content. Calculation of the ascorbic acid data on the dry weight basis eliminated the apparent differences due to location

DISCUSSION

It seems apparent that the leaves must be responsible for the synthesis of the ascorbic acid or its immediate precursors, since the fruit that was fully exposed to the sunlight was not significantly higher in ascorbic acid than the shaded fruit, and only when the whole plant was shaded did the ascorbic acid level fall greatly. These data confirm those of Hansen and Waldo (*2*) in this respect, although these investigators, as well as Ezell et al. (*1*), found more ascorbic acid in the fruit when the whole plant was exposed than when only the berries were shaded. It is possible that the difference in type or degree of shading may explain this disagreement. Hansen and Waldo used unbleached muslin bags, while Ezell and coworkers used brown manila paper bags. Since white parchment paper bags were used in the studies reported here more light probably reached the fruit and there may have been a temperature difference as well.

The 5- to 6-day lag in response of the ascorbic acid level of the ripe fruit to the amount of insolation would indicate that the synthesis of either ascorbic acid or its precursors is stimulated by sunlight before the table-ripe stage is reached. Possibly the lag period represents the time from cessation of translocation from the leaves to the fruit, or it may be the time necessary for the precursor, formation of which is stimulated by sunlight, to be transformed into ascorbic acid. The latter hypothesis receives support from the observation of Ezell and his coworkers that the ascorbic acid of strawberries that are picked

Table 2.—Effect of different locations on the ascorbic acid content of strawberries

Variety	Ascorbic acid			
	Fresh weight basis		Dry weight basis	
	Dunkirk fine sandy loam	Dunkirk silty clay loam	Dunkirk fine sandy loam	Dunkirk silty clay loam
	Mg. per 100 gm.	*Mg. per 100 gm.*	*Mg. per 100 gm.*	*Mg. per 100 gm.*
Sparkle	80.4	61.0	616	522
Fairfax	82.0	82.3	628	640
Catskill	88.1	69.8	708	694
Dresden	85.9	77.3	792	740
24248	88.5	64.5	718	556
25460	97.8	79.0	792	762
28340	52.9	55.0	500	530
28597	76.0	64.1	530	569
USDA 3414	77.2	74.7	558	599
			649	
Average	81.0	69.7		623
Z value for comparison of difference	1.21		0.40	
Odds that difference is significant	200:1		5.9:1*	

*Not significant.

before complete ripening continues to increase after separation from the plant.

That soil type may influence the composition of the strawberry fruit is demonstrated in the comparison of the fruit grown at the two locations. The difference noted in the ascorbic acid content of the strawberries was caused by a difference in the relative amounts of solids rather than differences in the amount of sunlight. The difference between the ascorbic acid content of the strawberries grown at the two locations when expressed on a fresh-weight basis disappears when these same results are expressed on a dry-weight basis. This indicates that other comparisons based solely upon a fresh weight basis may or may not be valid. There may or may not be a real difference in the amount of ascorbic acid which has been produced. Instead, there may simply be the same amount of ascorbic acid produced at each location, and the difference noted may have been caused by a difference in the water content of the strawberries, the silty clay loam having relatively more juicy berries than the plants on fine sandy loam.

The data presented in this paper confirm the observation of several other investigators that sunny days are essential for obtaining the maximum amount of ascorbic acid in strawberries. It is therefore to be expected that great variations in ascorbic acid content will occur in strawberries grown in different seasons and in different geographical locations.

SUMMARY

The synthesis of ascorbic acid in strawberries is stimulated by exposure to sunlight. With a 57-percent decrease in the amount of illumination during development and ripening, the ascorbic acid content averaged 36 percent lower. The data in this paper indicate that the degree of illumination of the leaves is the most important factor in this relationship.

The amount of ascorbic acid in the ripe fruit appears to correspond to the amount of illumination on the fifth or sixth day prior to harvest.

Differences in ascorbic acid content of strawberries on the fresh-weight basis occurred at different locations with the same amount of llumination. However, if the ascorbic acid is expressed on a dry-weight basis the differences due to environmental factors other than sunlight were insignificant.

LITERATURE CITED

(1) Ezell, B. D., Darrow, G. M., Wilcox, M. S., and Scott, D. H.
1947. ascorbic acid content of strawberries. Food Res. 12, 510–526.
(2) Hansen, E., and Waldo, G. F.
1944. ascorbic acid content of small fruits in relation to genetic and environmental factors. Food Res. 9: 453–461, illus.
(3) McCrory, S. A.
1946. vitamin c content of strawberries. N. and S. Dak. Hort. 19: 31–32, illus.
(4) Robinson, W. B., Lee, F. A., Slate, G. L., and Pederson, C. S.
1947. chemical composition and freezing adaptability of strawberries. N. Y. State Agr. Expt. Sta. Bul. 726, 14 pp., illus.
(5) ——— and Stotz, E.
1945. the indophenol-xylene extraction method for ascorbic acid and modifications for interfering substances. Jour. Biol. Chem. 160: 217–225.
(6) Sprague, V. G., and Williams, E. M.
1943. a simplified integrating light recorder for field use. Plant Physiol. 18: 131–133, illus.
(7) Student
1908. the probable error of a mean. Biometrika 6: 1–25, illus.
(8) ———
1925. new tables for testing the significance of observations. Metron (3) 5: [105]–108.

U. S. GOVERNMENT PRINTING OFFICE: 1949